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THE PROTOZOA OF THE HUMAN MOUTH

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From the standpoint of the parasitologist the vertebrate mouth is one of the main portals of entry for parasitic infections of the digestive tract and its morphological annexes, especially when there is added the additional factor of the mobile hand and opposable thumb, the use of implements and the infantile tendency to put anything the hand grasps into the mouth. In the case of civilized man, whose body is so generally protected elsewhere by clothing, shoes, hat and gloves, its relative importance becomes even greater.

The mouth of man is also one of the greatest areas for contact with the environment. Through the posterior nares the inhaled air and dust and germs collected from it on the surfaces of the nasal cavities has an indirect access to the buccal cavity. The food daily passed through the mouth, though weighing only several pounds, has passed through the hands of who knows how many hundreds of persons, sweating coolies rolling tea leaves in Chinese factories, laborers in Arabia, Sao Paulo or Limon washing out coffee beans, or Malays in Batavia gathering chocolate beans, negroes in Havana or Filipinos in Honolulu handling sugar, Mexicans picking oranges in Riverside, and so on through the long list of essentials and relishes that supply and embellish our daily menu. How far we should have to travel if we should attempt to subject the rest of our bodies to the geographical range of environment which has been in contact with the foods and drinks which we daily introduce into our mouths. Truly, how provincial is the rest of our corporal substance in comparison with the travelled versatility of our oral cavity!

How varied, too, are the substances which come in daily contact here with delicate mucus membranes. They range in temperature from below freezing to nearly boiling point and include both acids and bases, essential oils, fats and alkalis, sugars, salts, and proteins of the widest range. Even under the most rigorous treatment of any mode of physiotherapy no other part of our body receives daily so varied applications of the stimulating materials from the external world.

The mouth is also a region of no little mechanical shock and impact. Powerful muscles bring the teeth in contact with food which is ground

up and mixed with the saliva. The teeth upon which this impact is first received transmit the pressure to the delicate tissues which invest their imbedded surfaces, and thence to the bony alveolar sockets in which they rest. No other part of the body receives such an impact on so restricted a surface, except possibly the soles of the feet of the hobo or the athlete.

The mouth is also the arena of an exceptional amount of morphological change during the life of the individual, the only changes approaching it in range being the changes at adolescence which depend immediately upon the endocrine system. The emergence of the primary dentition, its resorption, evacuation, and replacement by the secondary dentition involves tissue growths and resorptions, readjustments of skeletal relations and modifications of muscular activity. The loss of teeth through decay also involves changes in the bony tissues in which the teeth are seated, so that occlusion and pressure are readjusted to the new conditions.

Dietary disturbances, notably those of a scorbutic type dependent upon vitamines or upon the acid-base balance in blood and in saliva also modify oral conditions and lead to disturbances in the gingivae and alveolar bone adjacent to the teeth. Pregnancy with its heavy drafts on the calcium salts of the mother often leads to bone resorption in the jaws and gives rise to the saying that each baby costs a tooth.

To these normal modifications and disturbances due to the cyclic changes of life and to functional vicissitudes there must also be added the lesions made in the delicate tissues of the gingivae in the course of mastication, by hard particles of the food, by toothpicks, or by the tooth brush. In addition impacted food between teeth affords a culture medium for bacteria and other organisms which either directly or through the agency of their excreta and toxins, weaken and eventually destroy the epithelial barricade and then permit the invasion of organisms into the lesion. It is in these various ways that the attachment of the infolded epithelium of the gingivae to the cementum is broken down and a lesion known as the gingival groove arises which becomes a seat of infection and destruction of the gingival and alveolar tissue.

The cooked and softened food of civilized life, the fermentation of carbohydrates, the access of infections from nasal sinuses, the promiscuous osculation formerly inflicted upon helpless infants, and still occasionally lapsed into in later life, the transfer of eating utensils from the mouth of parent to child, the tendency of children to suck their thumbs and put anything in their hands into their mouths, and droplet infection, all combine to increase the range of infections which in the course of a lifetime impinge upon the favorable seed bed found in the lesions of the gingival groove.

In view of these favoring conditions for the invasion of infections and for their maintenance when once established, it is not strange that a dental profession whose function it is to care for oral diseases should have been developed and that focal infections profoundly affecting general health should so often occur in connection with the teeth.

The reason why oral diseases are not more prevalent than they are may be found in the rapid transit of food through the mouth, the cleansing effect of normal saliva, and the ease with which lesions heal in the normal mouth.

It is not my purpose to deal with the varied aspects of oral infections or of peridontosis or other forms of oral disease, but to invite your attention to the parasitic infections of animal origin only. In contrast with the exceedingly varied bacterial infections which microscopic examination brings to light, namely, of filamentous bacteria, such as *Leptothrix* and *Actinomyces,* and aggregates seething masses of spiral and rod-like bacteria, and the smaller rod and coccus forms, the animal invaders are relatively few in number and are limited to two species, drawn respectively from the Rhizopoda and the Flagellata.

Both occur in a type of peridontal disease known as Rigg's disease or pyorrhea, although they have not been reported in all cases of this disease examined for them and each may occur in mouths in which a diagnosis of even an early stage of pyorrhea might be at least tentative.

Our knowledge of animal parasites and their relation to human disease is much less complete than those caused by bacterial parasites. This is due at least in part to the fact that diseases caused by animal parasites often though not always progress to a chronic phase in which a balance between host and parasite is reached without either attaining complete ascendancy over the other. Such diseases caused by animals are: malaria, chronic amoebiasis, filiariasis, intestinal flagellosis, many animal trypanosome infections, etc. On the other hand, bacterial infections are most likely to run an acute course with more rapidly developing and clear-cut symptoms, and speedy victory for either host or parasite. This results in the complete syndrome coming in a relatively short time under the observation of the physician, with consequent completeness of knowledge.

Animal parasites of the mouth have been but little studied by protozoologists until very recently, in spite of the fact that a considerable percentage of the adult population is infected with one or both of the common one-celled animals which occur in very close association with certain forms of gingival disease.

The two common parasites of the mouth, *Endamoeba gingivalis* and *Trichomonas buccalis,* will be dealt with in separate sections.

ENDAMOEBA GINGIVALIS (GROS) HISTORICAL INTRODUCTION

Gros (1849) first directed attention to the common amoeba of the human mouth by giving it a meager description and the name *Amoebea gengivalis*. It is of interest to note that this was the first parasitic amoeba of man to be discovered. Although the specific name *buccalis* has enjoyed wide usage there is no doubt but that Gros saw and drew with considerable accuracy the common amoeba of the mouth. His drawings show the characteristic large broad hyaline pseudopodia and the enclosed food vacuoles. His description is much less instructive than his drawings, and he suggests a comparison of the enclosed food vacuoles with those observed by Ehrenberg in the Infusoria to which the name *"Polygastrica"* was given by the latter investigator. Gros has thus corectly interpreted these as food vacuoles. The source and fate of these organisms interested him and he asked the question, *"Est-ce encore une génération spontanée?"*

Thirteen years later Steinberg (1862), ignorant of the work of Gros, dealt with the same organism at length in a thesis published in the Russian language. His work is of little value and it cannot be definitely ascertained whether or not he was working with the common amœba of the mouth because of his inadequate description and his failure to publish drawings.

Grassi (1879) reported in one brief paragraph without illustrations the existence of an organism from the human mouth which he called *Amoeba dentalis*. He mentioned that it was found in gingival inflammation and that it resembled the *"Amoeba coli"* of the human intestine. He later (Grassi, 1895) doubted his own observation and suggested that he had seen only salivary leucocytes.

In 1904 Prowazek, apparently ignorant of the work of the preceding observers, published an account of what he thought to be a new amoeba. It is not surprising that he gave the same specific name previously employed by Steinberg since the name *Entamoeba buccalis* is a logical one to apply to an organism in this habitat. Because of the wide circulation which this article received and because of Prowazek's standing in the field of protozoology his name was the accepted one previous to 1915.

Brumpt in 1913 called attention to the priority of the specific name given by Gros and referred the parasite to the genus *Entamoeba*, attributing this generic name to Leidy (1879), but the latter used the name *Endamoeba*, *Entamoeba* being its homonym. Smith, Middleton and Barrett (1915) appear to be the first to have used the generic name *Endamoeba* for *gingivalis*.

In July of 1914 Barrett made the significant announcement that he found *Endamoeba gingivalis* in nearly all cases of pyorrhea alveolaris

which he examined. He advanced the hypothesis that it was of etiological significance in this disease since emetin hydrochloride and other derivatives of ipecac seemed to be of therapeutic value.

In August of the same year (1914) Chiavaro, a student of Grassi, published a valuable contribution containing clinical data on 68 examinations for the presence of *Endamoeba gingivalis*. In 100% of 22 cases of classical pyorrhea with the production of macroscopic pus the parasite was found without exception. One of these cases after treatment with "medicine containing inorganic acids" did not yield amoebas in the single examination following treatment. In 20 cases of caries the amoebas were present in the cavities of 5 individuals and absent in 15. We cannot, however, be certain from his account that incipient pyorrhea might not have been present at the same time in the gums. In *materia alba* removed from the necks of teeth in 13 examinations 7 specimens contained amoebas and 6 contained no amoebas. In 7 samples of hard triturated tartar washed in running water there were no amoebas. In 2 specimens of "yellow-orange deposit" amoebas were found. They were present in the pus from one case of alveolar sequestrum and absent from the following: one specimen of "greenish deposit," one specimen of pus from a cutaneous fistula, and one gangrenous pulp. Chiavaro's conclusions based upon the preceding observations were that the amoeba instead of being pathogenic might be of value as a disinfecting agent, ingesting bacteria and cellular debris.

In the following month (September, 1914) Bass and Johns reported the presence of *Endamoeba gingivalis* in 86 cases of pyorrhea and their absence in many normal individuals. Beneficial results following the administration of emetin hydrochloride led them to assign a pathogenic role to this organism. In the following year they extended and confirmed these observations in 300 cases (Bass and Johns 1915a). In the same year (Bass and Johns 1915b) their observations and arguments were presented in the form of a book entitled Alveolodental Pyorrhea, which contained the gist of the affirmative evidence for their theories. In this work they continually assume that the parasites are living in and at the expense of the gingival and alveolar tissues without, however, showing histological evidence of such localization in the tissues. They state that amoebas were found to be most numerous at the extreme depth of the pocket. This is based upon evidence secured in the following way and in a single recorded case. Smears were made from various points on the surface of an extracted tooth from a case of pyorrhea. Amoebas were recovered in much the greatest numbers in a smear prepared from material removed from the surface of a ledge of subgingival tartar at a point nearest the extreme depth of the pocket. This observation has received little attention by subsequent workers but has been strikingly confirmed and extended in observations later to be described.

The use of ipecac derivatives in the treatment of pyorrhea became an almost universal practice among progressive dentists following the appearance of the important publications of Smith and Barrett and of Bass and Johns. The results furnish one of the most disappointing chapters in medical and dental history. It was not long before it became evident that emetin was not a specific for pyorrhea. This led to the abandonment of the procedure and also of the theory upon which the practice was based (Dobell and Laidlaw, 1926, and Howitt, 1926). The term "pyorrhea" had no sharply limited clinical definition, in consequence of which many reports were published on the presence of *Endamoeba gingivalis* in mouths that were considered by the observers to be free from pyorrhea (Williams et al, 1914-1915, and Mitchell, Culpepper and Ayer, 1916). Others reported their absence from cases considered to be pyorrhea. Thus in a relatively short time the theory was abandoned. Its fall was as rapid as its rise and fully as dramatic. It has been accepted on insecure and insufficient evidence but was rejected because of even weaker logic.

MORPHOLOGY

Several more or less brief morphological studies on *Endamoeba gingivalis* appeared early (Prowazek, 1904, Chiavaro, 1914, Smith, Middleton and Barrett, 1914, Bass and Johns, 1915, Smith and Barrett, 1915, Craig, 1916, Goodrich and Mosely, 1916). These deal chiefly with the appearance of the animal in the living state and the nature of the food inclusions. It was not until 1924 that a critical study of nuclear structure appeared. This investigation (Kofoid and Swezy, 1924) was prompted by the rather wide-spread opinion that arthritic symptoms are correlated with focal infections of the mouth. Having reported *Endamoeba dysenteriae (=E. histolytica)* in the bone marrow in cases of arthritis deformans it occurred to these authors that the mouth also might be an area of tissue localization of *E. dysenteriae* as well as of *E. gingivalis*. A detailed study of the cytological structure, particularly the nucleus, of *E. gingivalis* and of *E. dysenteriae* served effectively to separate these two species. Nuclear structure has proved to be our most dependable criterion for differentiation of species of amoebae (Kofoid, 1928).

In size most specimens of *Endamoeba gingivalis* range from 12 to 25 microns in diameter although specimens as small as 6 microns and as large as 60 microns have been described. In the living state the protoplasm is of a greenish, waxy, refractile appearance which distinguishes it from debris in which it may be partially or wholly imbedded. When in the rounded-up state the ectoplasm is not differentiated from the endoplasm. When in active locomotion the distinction between these layers is clear, the ectoplasm being remarkably hyaline and with a high refractive index. An external pellicle is readily visible. Pseudopodia

are usually few in number, broadly blunt, large, quite hyaline and are frequently thrust out with explosive rapidity resembling large blisters in appearance. When cold or moribund the pseudopodia may be formed very slowly, several minutes being consumed in the protrusion of a single pseudopodium. When lacking proper substratum, as in a hanging drop, a number of small conical pseudopodia may be formed and retracted if no favorable substratum is encountered. The endoplasm is very finely granular in life and when observed with dark field illumination is seen to contain a number of refractile granules not readily seen by transmitted light. The leucocytic food vacuoles are often seen in life, but the nucleus is seldom distinguishable.

Fixed specimens of *Endamoeba gingivalis* stained with Heidenhain's iron haematoxylin present a distinct and uniform pellicle. The ectoplasm is lightly stained and finely granular or faintly alveolar. The endoplasm contains more siderophile material and is not so distinctly differentiated from the ectoplasm as in life. The leucocytic food vacuoles stain intensely in many instances although not infrequently the contents of vacuoles in a late stage of digestion stain less intensely. The liquid siderophile contents of these vacuoles may be extruded through a small temporary opening to the exterior in long strands. This is not an artifact traceable to the smearing process in the preparation of the slide, but is a normal phase in the metabolism of the nucleoprotein as is evidenced by the frequency of its occurrence and by the fact that other amoebae may ingest these strands. It is our opinion that the quantity of nucleoprotein which can be metabolized by an amoeba is strictly limited. The remainder is probably not split further than into protein and nucleotides. The former is assimilated and the latter is sooner or later ejected. The amount of protein obtained in this manner from a given mass of nucleoprotein is relatively small and this accounts for the voracious appetite of what is a relatively sluggish animal in its normal habitat. From the shape of these strands it appears that they are spit out with explosive rapidity and rapidly eliminated. This accounts for the relative infrequence with which the process is observed. It must be admitted, however, that the ejected strands persist for a longer time than the ingestion of food particles, for the latter has seldom been observed and stages of ingestion are much scarcer in stained preparations than those of regurgitation of nucleotides.

Child (1926) also gave us a valuable contribution to the life history of this parasite in describing its reproduction by a complicated and orderly process of true mitosis.

FEEDING HABITS

Nowlin (1917) presented a purely hypothetical life cycle to account for periodic disappearance of the parasites assuming that they retreated into the tissues at such times. No fact has ever been brought to light

which supports this idea. The same author (Nowlin, 1917) presented another brief and inconclusive account of the food-taking habits of the organism, and of its behavior. Failing to observe stages in the actual ingestion of solid food particles she assumed that the process did not occur and that the organism fed chiefly by osmosis.

Goodey and Wellings (1917) attacked this problem of the food habits of *Endamoeba gingivalis*. Their conclusion differed from that voiced by von Prowazek (1904) who stated that the amoeba lived largely upon leucocytes. Goodey and Wellings concluded that not living leucocytes but only the denuded nuclei of degenerated leucocytes constituted the main source of food, together with various bacteria. They thus concluded that the amoeba could be considered as beneficial in destroying cellular debris and bacteria which might be harmful.

Although this conception has been widely accepted the work of Child (1926) has now demonstrated all stages in the ingestion of normal living leucocytes by the amoebas. The cytoplasm was observed to be stripped off and the nuclei of the leucocytes liquefied for food. The undigested remains were frequently extruded, a process previously observed by Kofoid and Swezy (1924). It should be stressed that the relative infrequence with which ingestion of leucocytes is observed may not mean that it is unusual but that it takes place with relative rapidity. The parasites are seldom seen in the process of food ingestion but are seen to eat leucocytes as frequently as any other food, and in full activity *in situ* are habitually gorged with leucocytes, and not with bacteria or red cells. *In vivo* it is evident from the work of Kofoid and Hinshaw (1929) that the life of the amoeba is quite intimately bound up with that of the filamentous bacteria of calculus.

PHYSIOLOGY AND CULTIVATION

A very significant contribution to the study of the amoeba of the mouth was that offered by Howitt (1925), who was first to successfully cultivate this parasite *in vitro*, although Drbohlav (1925) published first. Of particular significance is her observation that when living *in vitro* the organism ingests red blood cells, preferably human erythrocytes. Furthermore, the organism possesses the ability to haemolyse these cells even when not ingested. This suggests a relationship to *Endamoeba dysenteriae* which it so closely resembles morphologically. Howitt (1926) also observed the ingestion of living leucocytes in her cultures, verifying the observation of Child (1926) who saw the same phenomenon *in vivo*.

The amoeba is positively chemotactic for leucocytes and erythrocytes, ingesting both in cultures and both *in vivo*, though the former are abundant and the latter absent in a typical pyorrheal lesion. The formation of giant cells about the amoeba (Child, 1926) indicates clearly the

production of chemotactic substances according to the view of Wells (1925). This makes it possible that the amoeba is pyogenic. The relative pyogenic activities of these Protozoa and the pyogenic bacteria found in the actively suppurating pyorrhea pockets cannot be well ascertained. Hinshaw and Simonton (1928) determined that the amoeba is never present in the absence of a leucocytic exudate, while bacteria of many kinds are found about gums that are not inflamed. It is entirely possible that the extruded nucleotides constitute at least a portion of the chemotactic substances. Nucleinic acid is positively chemotactic (Wells, 1925). *In vitro* human red blood cells are attracted to *Endamoeba gingivalis*. Howitt (1925) observed from 20 to 30 red blood cells attached to a single amoeba in cultures with red cells. Of 274 amoebas counted 70 were found to contain from one to six erythrocytes within the endoplasm. She also observed the extracellular haemolysis of human red blood cells when touching or lying close to amoebas. All this suggests powerful intracellular and extracellular enzymes capable of attacking human cells and tissues.

Howitt (1925) showed that *E. gingivalis* in mixed bacterial cultures is able to withstand a wide range of hydrogen ion concentration, the initial P_H for optimum growth lying between P_H 7.0 and P_H 7.8. The growth curve of the amoebae in mixed bacterial culture follows that of the bacterial curve. She also found that *E. gingivalis* is able to exist and multiply in the presence of cod liver oil, of glucose in small concentrations, and of certain proteins, peptones and amino acids. Howitt (1926) demonstrated the effect of certain drugs and dyes upon *E. gingivalis*. She found that stovarsol is the most effective of the substances used *in vitro* against cultures of *E. gingivalis* and yatren the least effective; that arsenical compounds are more toxic than the non-arsenicals; that emetin hydrochloride, although somewhat toxic for the amoebae, is nevertheless not specifically effective *in vitro*; and that of the dyes, acriflavine and gentian violet, the former can be used only in the high dilutions because of its toxicity, while the latter may be used in very much stronger concentrations but without greatly hindering the amoebic growth. Howitt (1926) in a later paper showed that *E. gingivalis* will thrive in the presence of filtered human saliva, provided a solid substrate of coagulated egg is present. The amoebae were unable to withstand contact with human gastric juice containing the normal amount of acid, but would survive for several hours in fluid lacking in or with a reduced amount of HCl. The organisms rapidly explode in the presence of human bile, but merely shrink up and gradually degenerate when in contact with concentrated bile from the cat or the guinea pig.

Koch (1926) working with such dyes as acid fuchsin, phenosafranin, brilliant green, basic fuchsin, ethyl violet, acridine orange, acriflavine and

many others, either singly or in mixtures, was able to demonstrate the selective action of some of these dyes and dye mixtures. In many cases a bacteriostatic effect was obtained without toxicity to the protein of the amoeboid cell. This action resulted in the prolongation of the life of the culture, so that, without transplanting or changing the medium in any way, *E. gingivalis* remained alive and motile for 12 days *in vitro*. She is of the opinion that *E. gingivalis* can live in culture with bacteria in very small numbers. Reproduction of bacteria is not necessary for the growth of *E. gingivalis* and it is considered not impossible that bacteria are unnecessary for growth, other food material being supplied. Koch suggests tricresol 1-100 and formaldehyde 1-20 as effective disinfectants for killing cultures and general sterilization. The dyes which were amoebicidal in high dilution were not injurious to the gum tissue of normal rabbits and may probably be used as oral antiseptics. Koch (1927) obtained interesting results from experiments dealing with the relation of moisture and temperature to the viability of *E. gingivalis in vitro*. It was found that the amoeba is, apparently, remarkably resistant to temperatures lower than the optimum. *E. gingivalis* will remain alive after being exposed to a temperature of 0° Cent., for 18 hours, and revives in culture. The amoeba will survive temperatures of 20° to 40° Cent., that is from room temperature to a little above body temperature, indefinitely, but is rapidly killed at 55° C. and will not withstand temperatures between this and 45° Cent. for any great length of time. Through experiments upon the effect of drying culture material containing motile *E. gingivalis* it was found that the organism is unable to withstand a total absence of moisture, but survived for a short time after the circumambient fluid had disappeared. If the great resistance to low temperatures and the great resistance to drying are considered together, it may be concluded that *E. gingivalis* is easily transmitted by "droplet infection" and that any minute amount of saliva, carrying the organisms, even though it be microscopic, may be infectious if not completely dry. It is possible then to spread the organisms by the use of the common drinking cup, spoon, and like utensil, as well as by direct contact. Encystment has never been seen, either *in vivo* or in cultures of any age.

Howitt's (1925) modification of the Locke-egg slant-albumen medium of Boeck and Drbohlav (1925) was used as the routine medium for the cultivation of *E. gingivalis*. Coagulated egg slants containing a small amount of Locke's solution were used as a solid base and to these slants was added the Locke's albumen solution.

Gingival exudate was removed from the mouths of pyorrhea cases with a straight shafted dental scaler and introduced into tubes of Locke's egg-albumen previously heated to 37.5 C. All cultures were incubated for 48 hours at body temperature and subsequently examined for the presence of amoebae.

Several strains of *E. gingivalis* have been cultivated in my laboratory for extended periods, one strain being maintained under artificial cultivation for 33 months. Each culture is transplanted every 48 hours and kept at body temperature. There is no reason to doubt that cultures of the amoebae can be prolonged indefinitely if proper care is maintained.

Recently Johnstone (MSS) has shown that *E. gingivalis* is capable of ingesting starch grains. A small amount of starch added to a culture of *E. gingivalis* in Locke's egg-albumen prolongs the life of the culture as long as 10 days, causes an increase in size of nearly 50 per cent; greatly increases the rate of division and the number of amoebae, causes a peculiar disarrangement and great increase in quantity of the nuclear chromatin and a production of multinucleate amoebae.

RELATION OF *ENDAMOEBA GINGIVALIS* TO CLINICAL CONDITIONS

The work of Chiavaro (1914) referred to above is the first tabulated account of the conditions in which the amoeba of the mouth is found. Unfortunately he was more interested in the pathology of the tooth itself than in that of the gingivae. Most observers have since made reference to the amoeba in relation to the presence or absence of pyorrhea. (Bass and Johns, Smith and Barrett, etc.)

Since a universally accepted definition of pyorrhea is lacking Hinshaw and Simonton (1928) attempted to correlate amoebic infection with objective symptoms expressed in a quantitative way in 357 cases. Their figures are primarily based upon data obtained from young individuals (students) and hence could not be expected to be duplicated on similar fractions of the general population. There are very distinct advantages in the use of a fairly uniform group, especially if they are young.

In their conclusions the statement is made that amoebae were never found in the strictly normal mouth and were invariably found in typical and inflammatory pyorrhea from its incipiency until its termination, thus substantiating the early claim of Bass and Johns and Smith and Bassett. The existence and extension of gingival pockets is an invariable accompaniment of the inflammatory type. The amoebae were always found in the well kept mouths of these young persons when pockets were found with a depth of 2 mm. or more. Commonly but not invariably the gum margin had receded. Microscopic pus could always be demonstrated even when there was no gross pus at all and relatively little reddening of the gums. A purplish venous stasis characterizes more advanced cases. The gingivae are not always hypertrophied. Little significance could be associated with the presence or absence of calculus because most of the patients had had it removed after frequent intervals.

Large amounts of detritus seemed to be inhibitory to the growth of amoebae in the mouth. This, together with experiments in the cul-

ture tube, demonstrates that the amoebae will not tolerate association with the putrefactive bacteria of decaying debris and must not be thought of as scavengers or organisms of decay.

No correlation was noted with the presence of caries or a history of caries. The saliva was usually plentiful or of a physical constitution which caused the production of a glairy film over the mucous membrane of the mouth. Patients who took good care of their mouths were just as likely to bear the infection. Luetics who had received treatments with neosalvarsan and mercury did not show any evidence that the parasites are killed *in vivo* by these treatments.

With increasing age the incidence of infection rapidly increases until those over 40 years of age have an incidence of 75 per cent or more. Nearly 50 per cent of all persons studied possessed the infection in spite of the preponderance of young patients.

LOCALIZATION IN THE PYORRHEA POCKET

It is surprising that *Endamoeba gingivalis* was not reported from histological sections of dental material at an earlier date. This is undoubtedly due to the technical difficulties in the way of making sections of tissue with tooth, bone, calculus and soft tissue *in situ* in proper relations. It remained for Kofoid and Hinshaw (1929) to first describe the location and associations of the parasite *in situ* in the pyorrhea pocket. In confirmation of the work of Naeslund (1925) the subgingival calculus was found to consist of the calcified strands of filamentous bacteria of the *Leptothrix* and *Actinomyces* groups. Naeslund offers convincing evidence that these organisms are the etiological factor in the formation of this deposit. Kofoid and Hinshaw find hundreds of amoebas *(E. gingivalis)* clustered among the terminal strands of these vegetations and forming a veritable palisadal covering to the calculus where the latter impinges upon the tissue of the gum.

The amoebae extend to the extreme depth of the pyorrhea pocket, and are most numerous in the deeper levels about the area of most extensive deposit of the calculus. This substantiates the earlier findings of Bass and Johns (1915) that the parasites are most numerous in the depth of the pyorrhea pocket.

The distribution as above briefly outlined is very significant and suggestive of a correlation between parasitic invasions including that of amoeba into the gingival groove and the diseased condition which is found in typical pyorrhea of the inflammatory type, with calculus of the serumal or black tartar type, loosening of the otherwise healthy tooth in its socket by reason of bone absorption and an abundant flow of pus. There are other forms of oral disease involving diseased gums, and loosening of the teeth, which may be and often are called pyorrhea, in the dental literature. More precise clinical definitions of the various

forms of paradentosis are needed to distinguish the inflammatory type, if possible, from the others.

In the first place the amoebae are very numerous and form a heavy layer in the outer free filaments covering the surface of the solid serumal tartar. They are most abundant about the point or angle in the deeper part of the pocket where the tartar is thickest.

(2) They extend some distance below the tartar adjacent to the cementum.

(3) They do not invade the gingival tissue adjacent to the mass of tartar but lie in the fringe of bacteria over against the swarming leucocytes upon whose nuclei they are feeding.

(4) The bony tissue between the teeth and below the level of tartar formation is extensively resorbed without accompanying evidence of infection by either bacteria or amoebae.

The picture thus presented is that of an association of organisms occupying a peculiar ecologic niche in the subgingival groove. It involves an infection by *Leptothrix* or other filamentous bacteria attached to the side of the tooth. The serumal tartar composed largely of calcium carbonate and calcium phosphate is deposited about the bases of these filaments. The bacteria themselves belong to the systematic group of bacteria which are associated with the deposition of lime salts in mineral springs and hot springs widely in nature.

The second organism, *Endamoeba gingivalis,* is confined in these sections in which the natural relations are undisturbed except slightly by decalcification to the fringe of the bacteria projecting beyond the calculus. In this position the amoebae are brought in direct contact with the outpouring stream of leucocytes upon whose nuclei they feed. The partially digested liquefied chromatin ejected from their food vacuoles contributes nucleoproteins or their derivatives rich in compounds of phosphorus available for deposition in the tartar as calcium phosphate. The bone of the sockets of the adjacent teeth is being resorbed near to the site of deposition of tartar containing calcium carbonate and phosphate. How much this process contributes to the tartar and what mechanism, if any, operates to bring about such a transfer of substance is at present wholly a matter of conjecture.

Evidences of intensity of the reaction of the host to the stimulus of this combination of bacteria, amoebae and tartar and probably also to accompanying bacterial agents is indicated by the wall of leucocytes piled up against the foreign invaders and by the many supporting leucocytes on their way through the adjacent layer of epithelial cells. These leucocytes contribute to the pus which flows from the pyorrheal pocket.

The accumulation of leucocytes and resulting flow of pus has a similarity to the flow of pus which follows amoebic invasion of the

intestinal epithelium, but there is lacking in the pyorrheal abscess the mucus and blood which characterize a dysenteric stool. The intense local reaction which results in the continuous emigrations of leucocytes across the epithelian barricade and the development of the abscess are clearly indicative of an inflammatory process. The total mass of foreign protein is very much greater in the amoebae than in the bacteria and they lie in immediate contact with the reacting tissue of the host. They also tend to precede the downward invasian of tartar on the side of the tooth. Their action is clearly not that of a scavenger and the reaction of the host to the invading complex is typically inflammatory.

Dental instrumentation so widely used in the treatment of pyorrhea removes this association from its ecologic niche by scaling of the tartar and bacteria and removing some at least of the amoebae. Gingival surgery which removes not only the tartar but also the diseased wall of the pocket and exposes the cementum to the normal fluids of the mouth and so to cleansing, likewise serves to check the ravages of pyorrhea. Both of these more or less successful therapeutic measures are directed against this complex of bacteria, tartar and amoebae.

THE EXPERIMENTAL INFECTION OF ANIMALS WITH *ENDAMOEBA GINGIVALIS* OF THE HUMAN MOUTH

The experimental work done in this laboratory has been with dogs and monkeys, the latter represented by the two species *Macacus rhesus* and *Macacus cynomolgus*.

Previous to the inoculation of each dog a thorough examination of the mouth was made. In selecting favorable dogs at the pound only those that were in good physical condition and had good or fair mouths were taken. The first group of dogs selected had mouths that showed reddening of the gums, gingivitis and gingival pockets. Each dog was submitted to a detailed examination, the general tone of the whole mouth and the condition of each tooth with its adjacent tissues was noted. Natural color photographs and roentgenographs of several of the dogs were taken.

Prior to inoculation the dogs were examined for possible protozoan contaminations. Cultures in L.E.A. and gingival exudate smears stained by the ordinary iron-haematoxylin method were made. A straight shafted dental scaler proved to be the best instrument for removing the material. All cultures were incubated for 48 hours at 37.5° Cent.

In order to avoid any possibility of overlooking a focus of infection the whole mouth was examined. Those dogs that were infected with *Trichomonas buccalis* were sent back to the pound as well as those that had a type of spirochaetal infection resembling Vincent's angina. No dogs with natural infections of amoebae were found.

To prevent chilling, the cultures of *E. gingivalis* used for inoculation obtained at San Quentin and San Francisco were transported from the above places in pockets sewed on the inside of the vest. The tubes were incubated for 48 hours at 37.5° Cent. and then examined for the presence of amoebae. We are of the opinion that it is better to use fresh cultures for each inoculation in preference to cultures that have been maintained *in vitro* for long periods. The dogs were anaesthetized before inoculation. A syringe was found unsatisfactory for inoculation of the material from the cultures because the small particles of egg that are always present clog the needle. A Pasteur pipette was used to transfer the amoebae from the bottom of the tube to the teeth, and as the material was dropped on the tooth the small particles of egg and debris (to which the amoebae cling) were forced under the gingiva with a curved dental instrument. The upper teeth only were inoculated and the lower kept as controls. Subsequent cultures and smears taken showed whether or not the inoculation had been successful. It was found better to refrain from removing any gingival exudate for one or two months after inoculation to avoid possibility of destroying a focus of infection. The one monkey inoculated could be handled without being anaesthetized.

Direct smears of gingival exudate from the human, dog and monkey mouth and smears from culture material were made by moistening the material in sheep serum and spreading it thinly on glass slides. The wet preparation was fixed in hot Schaudinn's fluid and stained by the usual iron-haematoxylin method.

Nile blue sulfate (Grübler) affords an excellent opportunity for the observation of motile amoebae from cultures. If the amoebae are grown in a dilution of 1-40,000 in L.E.A., many striking characteristics can be noted. The inclusions within the amoeba are stained a distinct blue so that the endoplasm and ectoplasm are clearly defined and pseudopodial movement is easily observed. A simpler method is to add a drop of Nile blue, made up in Locke's solution in a dilution of 1-50,000 to a slide preparation of the culture material. This gives a more intense stain than the above method. The nucleus is clearly seen and the details of movement can be followed with ease.

With the exception of the one dog infected by Hinshaw (1928) with *Endamoeba gingivalis* of the human mouth, this parasite has not been successfully maintained heretofore in experimental animals. We have infected five dogs with this amoeba and examinations up to the present time confirm their presence. As already stated, each dog is submitted to a thorough examination, covering extended periods, to demonstrate the absence of a possible spontaneous infection of oral amoebae. Of all the dogs examined, none showed any spontaneous infection of oral amoebae. All of the dogs infected and the controls are kept under

properly balanced diets and the best living conditions. A total of eleven dogs were inoculated with culture material containing *E. gingivalis* and in five of them the parasite still continues to multiply. It was found that the infection was successful only on those dogs which showed gingival inflammation, pocket formation or loose gingivae at the time of inoculation. The dogs with excellent mouths did not become infected.

It was our original intention when the work first began to try inoculating dogs and monkeys with *E. gingivalis* from the human mouth, but with the subsequent examinations of the monkeys' mouths it was found that all of our monkeys were parasitized with a species of amoeba identical with the human form of *E. gingivalis*. The monkeys used in our experiments were *Macacus rhesus* and *Macacus cynomolgus*. Cultures and smears were made of the gingival exudate for purposes of routine examinations and study. The results of these examinations showed that all of the monkeys were parasitized with this amoeba, some more heavily than others. The condition of those monkeys showing the heaviest infections closely resembled pyorrhea of man.

Monkey 12 was inoculated with *E. gingivalis* of man with the possible hope of a differentiation being established between the two forms, but upon examination of the slides all of the amoebae were morphologically identical and it was impossible to determine whether or not the human form had become infective.

DOUBTFUL SPECIES

It has been claimed by many that there is more than one species of amoebae found in the oral and pharyngeal regions of man, but the majority of workers today believe that *E. gingivalis* is the only amoebic parasite in these regions. The forms described as *Entamoeba maxillaris* Kartulis (1893), *Entamoeba kartulisi* Doflein (1911), *Amoeba pulmonalis* Artault (1898), *Entamoeba pulmonalis* Brumpt (1913), *E. pyogenes* Verdun and Bruyant (1907), *E. confusa* Craig (1916), and *E. macrohyalina* Tibaldi (1920) are probably identical with *E. gingivalis*, although it may be that *E. maxillaris* Kartulis is *E. dysenteriae*. Craig (1926) withdraws his species *E. confusa* (1916) stating that the amoebae he described were merely the smallest forms of *E. gingivalis*. In 1920 Tibaldi described an amoeba obtained from the tonsil. This probably is no other than *E. gingivalis* which has been shown by Smith, Middleton and Barrett (1914) to invade inflamed tonsils. As suggested by Kofoid and Swezy (1924) the contractile vacuole, reported by Tibaldi, may have been only a discharging food vacuole. The many names that have been given are probably the results of observations on degenerate amoebae, as has been the case with *E. dysenteriae* and other intestinal forms. It has been suggested that *E. dysenteriae* may invade

the mouth, and that it is identical with *E. gingivalis,* but this has been conclusively shown not to be the case by Kofoid and Swezy (1924), a conclusion later proposed by Wenyon (1926). The absence of evidence of tissue invasion by *E. gingivalis* and the deep abscess in which *E. maxillaris* was reported render its identification with *E. gingivalis* less convincing.

Trichomonas buccalis

Trichomonas buccalis is a very common animal parasite of the gingivals ulcus of diseased mouths of man and the dog. It belongs to the primitive class of one-celled animals commonly called flagellates, so named because of their method of locomotion, by means of long slender whip-like flagella. Very little was known about this very common parasite until within the last two years.

No critical morphological account of *Trichomonas buccalis* has appeared in the literature previous to that of Hinshaw (1926). Mitotic division was reported then for the first time in the species. The prevalence of this widespread infection of the human mouth was not realized before the work of Hinshaw (1926). Nor had experimental inoculation of animals been previously accomplished. Cultivation *in vitro* was accomplished by Lynch (1915), Ohira and Noguchi (1917), and Hogue (1926). These were, however, brief reports permitting the drawing of no general conclusions. A more extensive work on observations *in vitro* is that of Hinshaw (1927).

HISTORICAL REVIEW

O. F. Müller in 1773 described a pear-shaped organism occurring in the human mouth to which he gave the name *Cercaria tenax,* thus identifying it with a genus now known to represent a developmental stage in the life cycle of certain trematode worms. Since no organism other than *Trichomonas buccalis* occurs in the human mouth which even remotely resembles a cercaria, it is probable that Müller (Kofoid, 1920) was first to record this infection.

According to Doflein (1911), Steinberg (1862) was next to deal with this flagellate. He named three species from the mouth, *Trichomonas elongata, T. caudata,* and *T. flagellata.* His publication is in an inaccessible journal in the Russian language. There is no doubt but that his conclusions regarding the number of species were incorrect, for no subsequent investigator has succeeded in confirming them. The differences between his three alleged species were entirely quantitative characteristics, such as shape and size. He detected no qualitative differences.

Several observers have regarded *Trichomonas buccalis* as identical with the similar organism occurring in the human intestine, *T. hominis* (Prowazek, 1904; Doflein, 1911; Ohira and Noguchi, 1917). It is

more closely related to the *T. vaginalis* of the human vagina described by Donne (1837) than to any other species of the genus. This latter is the type species of the genus and if the three species be regarded as one the name *T. vaginalis* must be used. However, the cytological work of Hegner (1925) on the latter species when compared with our own work on *T. buccalis* (Hinshaw, 1926) shows sufficiently striking differences to warrant specific separation. Unpublished cultural work done by Johnstone (MSS) indicates that these two latter species are specifically distinct.

It is difficult to classify the trichomonads reported from cases of gastric cancer and still more difficult to place those reported from the oesophagus (Doflein, 1911). It is probable that these are derived from the mouth, as a descending infection is easier to imagine than an ascending one, especially since the *Trichomonas hominis* of the intestine normally lives in the lower portions of the alimentary canal. However, an ascending infection is not impossible. In this connection should be mentioned trichomonads from the sputum and lungs of persons afflicted with tubercular pulmonary phthisis, pulmonary gangrene, and putrid bronchitis (Schmidt, 1895). Schmidt gave the name *T. pulmonalis* to these forms, but most reviewers have regarded them as identical with *T. buccalis*.

It was not until 1917 that the accepted specific name was given for the trichomonad flagellate of the mouth. Goodey (1917) gave the name *Tetratrichomonas buccalis*. This paper was appended to a joint paper by Goodey and Wellings (1917) and most reviewers have ascribed the authorship of the species to these two, whereas the technical authority for the species is Goodey alone.

Little evidence is recorded in the literature with regard to the pathogenicity of these parasites. The intestinal form is often considered to be harmless. Some consider the flagellate of the vagina to be pathogenic, being found commonly in leucorrhea when the discharge is acid in reaction. Lynch (1915) regarded trichomonads found in the mouth and vagina as pathogenic. Seven years later (Lynch, 1922) he expressed considerable doubt as to their role in diseases of the intestine, genital tract, and buccal cavity.

OCCURRENCE

This parasite of the human mouth is usually considered to be relatively rare and of sporadic occurrence if one may judge by the infrequent references in the literature of the microbiology of the mouth. This widespread conception is based largely upon inadequate technique. Dried smears stained with eosin-methylene blue or other stain have been depended upon too frequently to give us an idea of the protozoology of the buccal cavity. *Trichomonas buccalis* is an extremely delicate

organism and is not readily demonstrated by such technique. Even the use of carefully fixed wet smears stained well with Heidenhain's iron haematoxylin cannot be expected to reveal more than 10% of the cases of infection. Examination of material in the living state is more trustworthy than either of the above methods, but many sources of error occur here. Even to the experienced eye an active trichomonad, viewed in the seething mass of cells and bacteria with which it is associated in the mouth, may be readily mistaken for a salivary corpuscle agitated by actively motile spirochaetes or bacteria. We have shown (Hinshaw, 1926) that these parasites tend to bury themselves in the solid debris with which they are associated in the mouth and hence escape detection. We have found that the inoculation of Boeck's medium with a small amount of the pyorrheal pus or interproximal debris followed by incubation at 37° C. for 48 hours reveals nearly 100% more cases of infection than any other means.

To find nearly 90% of the cases of advanced pyorrhea examined in San Quentin Prison infected with *Trichomonas buccalis* was startling (Hinshaw, 1926). It might appear that this represents an epidemic condition within the walls of the prison. This cannot be the case, for approximately one half of all those examined were cultured at the time of the dental examination given each prisoner upon being admitted to the institution. The incidence of trichomonad infection of pathological mouths was fully as high in those just entering the prison as in those who had resided there for months and years. It will be observed that the incidence of typical advanced pyorrhea in this institution is unusually high. It is significantly lower in those who were just entering. The crowded conditions with multiplicity of contacts, together possibly with deficient diets are in part responsible for this contrast. The age factor is an extremely important one and residents of the institution are on the average older than those just entering.

Our examination of persons outside San Quentin has been largely confined to students. We met with few cases of advanced pyorrhea among these young persons, so it is not surprising that infections with *Trichomonas buccalis* were seldom found. Only three such cases were observed. One was a male student, 31 years of age, presenting a bad case of pyorrhea. Another was a female student, 47 years of age, with extensive pockets and receded gingivae. She reported frequent periodic attacks of intense inflammation of the gums. The third case was in a male 36 years of age. The gums were receded and some shallow pockets were present. He reported that he had formerly had several rather extensive pockets, but that careful instrumentation had recently improved the condition considerably. This infection was evidently residual from the former condition of more advanced pyorrhea. Smears from this case showed considerable quantities of pus although the gums

were macroscopically of healthy appearance. The man stated that he always brushed his teeth thoroughly five times a day.

Age is an important factor predisposing to infection with this parasite. Although the greater numbers of our patients were between the ages of 17 and 26 years, with the mode at 22, the increased incidence in older age groups is striking. The youngest one-fourth of the population group studied had an incidence of only 3.63%, and the older groups 12.47%, 12.36%, 42.1% respectively. Over 40% of those whom we examined who were over 30 years of age were found to possess this parasite formerly thought to be rarely present in the human mouth!

MORPHOLOGY AND REPRODUCTION

Most published figures of *Trichomonas buccalis* are inadequate and often inaccurate. The nucleus is usually represented as a solidly staining structure, whereas when properly stained considerable internal structure can be made out. The nucleus of cultural forms is usually composed of several rounded granules. This organism has an integrated neuromotor system consisting of four flagella arising from a three-lobed blepharoplast which also gives rise to a chromatic basal rod and an axostyle. This axostyle takes basic stains as does that of *T. vaginalis* of the human vagina, whereas other trichomonads have an axostyle which is hyaline and unstained in mounted preparations.

Trichomonas buccalis reproduces by simple, asexual fission preceded by a true process of mitosis involving the appearance of three morphologically distinct chromosomes. The flagella are evenly divided between the two daughters and the missing ones are regenerated. The axostyles and chromatic basal rods are resorbed during the process of mitosis and regenerated in each of the daughter animals.

EXPERIMENTAL INFECTION OF ANIMALS WITH *TRICHOMONAS BUCCALIS*

As with *Endamoeba gingivalis* this animal parasite of the human mouth has not heretofore been maintained in experimental animals. The dogs and monkeys used for experimental purposes were submitted to a thorough examination over extended periods to preclude any possibility of natural infection with this parasite. We found that of 29 dogs examined for *Trichomonas buccalis* only 6 bore the infection spontaneously. Hegner and Ratcliffe (1927) reported the finding of this parasite in 22 of 23 dogs examined. They are of the opinion that the species found in the mouth of the dogs is a distinct one and that the infection was spread by association when the dogs were allowed to run together. Hinshaw (1928) successfully inoculated an old dog possessing an advanced case of gingivitis with material from a culture of *T. buccalis*.

The parasite continued to multiply in the dog until he was sacrificed fourteen and one-half months later.

Not any of the nine monkeys examined were found to harbor a trichomonad parasite of the mouth. Five of the monkeys were inoculated with material from cultures of *Trichomonas buccalis* of the human mouth and subsequent examinations showed that in three of them the infection was successful.

Cultures were taken for a period of three weeks from the vaginas of two monkeys to determine the presence of a trichomonad parasite. When it was found that no such parasite was present in either of the monkeys culture material of *T. buccalis* from the human mouth was introduced into the vagina of each monkey. Four days later a second introduction of material was made. Subsequent culture examination showed that the infection had not taken. Dog number 10 was inoculated subgingivally with material from a culture of *Trichomonas vaginalis* from the human vagina. Several examinations following the inoculation of this parasite into the mouth of the dog proved negative.

In conclusion it may be stated that a definite correlation exists between the Protozoa of the mouth and the inflammatory type of pyorrhea alveolaris. Experimental evidence thus far available is indicative of the possibilities of producing in experimental animals clinical conditions similar to those found in man. The time factor in experimental work with animals is as important as in the case of human infection.

The conditions revealed by the study of the protozoan infections of the mouth are instructive in the matter of the biological concept of disease and its origin and progress.

Pathology and the etiology of disease in the past has often stressed the importance of the single etiological factor and attention has been focused in research on the demonstration of this factor according to the principles so ably laid down by Koch.

Modern atomic physics, endocrinology, and of late even genetics are modifying our biological philosophy, so that we are tending more to conceive the organism as an interacting system of parts, all of which are or may be concerned in the phenomena of life. Perhaps also in our philosophy of disease we should likewise seek to find and to define more clearly the other factors which interact with the so-called single etiological factor and play a not unimportant part in emergence of conditions which we designate as disease. The carrier phase and the occurrence of so-called etiological factors in persons not presenting symptoms of the disease at the time of examination are significant in this connection.

In the case of pyorrhea we have in addition to the two seemingly constant attendants upon typical inflammatory pyorrhea, namely the filamentous bacteria and *Endamoeba gingivalis*, other factors, such as the spiral

bacteria, the mechanical lesions, the salivary components, the dietary sequelae, the carrier stage of infection, and the hereditary diathesis of the host. The biologist who is able to expert the books of this interacting system of component parts and present a balance sheet of profit and loss to each of the contributors has a herculean if not impossible task. However, the dental practitioner, the oral surgeon, and the dental hygienist may profit by the exhibit of the picture of infection and each may direct his therapy and preventive procedures against this array of oral invaders and functional disorders with some better hope of success with this picture before him, than without it.

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Literature Cited

Artault, S. 1898.—Flore et faune des cavernes pulmonaires. Arch. de Paras., 1: 217-307, pl. 1, 1 chart.

Barrett, M. T. 1914.—The Protozoa of the mouth in relation to *Pyorrhea alveolaris*. Dent. Cosmos, 56: 948-953.

Bass, C. C. and Johns, F. M. 1914.—Unpublished report to the Orleans Parish Medical Society, September, 1914.

1915a.—*Pyorrhea dentalis* and *alveolaris*. Specific cause and treatment. Jour. Am. Med. Assoc., 64: 553-558, 9 figs. in text.

1915b.—Alveolodental pyorrhea (Philadelphia, Saunders). 167 pp., 1 pl., 42 figs. in text.

Boeck, W. C. and Drbohlav, J. J. 1925.—The cultivation of *Endamoeba histolytica*. Am. Jour. Hyg., 5: 371-407, 3 pls.

Brumpt, E. 1913.—Précis de Parasitologie. (ed. 2, Paris, Masson.) xxiv + 1011 pp., 4 pls., 698 figs. in text.

Chiavaro, A. 1914.—Researches upon *Entamoeba buccalis*. Dent. Rev., 28: 1122-1135, 10 figs. on one plate.

Child, H. J. 1926.—Studies on the ingestion of leucocytes, and on mitosis, in *Endamoeba gingivalis*. Univ. Calif. Publ. Zool., 28: 251-284, pls. 25-29, 9 figs. in text.

Craig, C. F. 1916.—Observations upon Endamoebae of the mouth. I. *Endamoeba gingivalis* (buccalis). Jour. Inf. Dis., 18: 220-238, pl. 6.

1926.—A manual of the parasitic Protozoa of man. (Philadelphia and London, Lippincott.) vii + 569 pp., 95 figs. in text.

Dobell, C. and Laidlaw, P. P. 1926.—The action of ipecacuanha alkaloids on *Entamoeba histolytica* and some other entozoic amoebae in culture. Parasitology, 18: 206-223.

Doflein, F. 1911.—Lehrbuch der Protozoenkunde. (ed. 3) (Jena, Fischer.) xii + 1043 pp., 951 figs. in text.

Donne, A. 1837.—Recherches microscopiques sur la nature des mucus et la matiére des divers écoulements des organs genito-urinaires chez l'homme et chez la femme. (Paris, Chevalier). pp. iv + 71, pl. 1.

Drbohlav, J. J. 1925.—Culture *d'Entamoeba gingivalis* (Gros 1849) Brumpt 1913. Ann. Parasit., 3: 361-363.

Goodey, T. 1917.—A note on the trichomonad flagellate *Tetratrichomonas buccalis*, n. sp. Parasitology, 9: 554-557, pl. 22.

Goodey, T. and Wellings, A. W. 1917.—Observations on Entamoeba gingivalis from the human mouth, with a note on the trichomonad flagellate *Tetratrichomonas buccalis*, n. sp. Parasitology, 9: 537-559, pls. 20-22.

Goodrich, H. P. and Moseley, M. 1916.—On certain parasites of the mouth in cases of pyorrhoea. Jour. Roy. Micr. Soc., 1916, 513-527, pls. 12-17.

Grassi, B. 1879.—Dei Protozoi parassitti e specialmente de quelli che sono nell uomo. Gazz. Med. Ital. Lomb., 39: 445-448.

1895.—Cf. Railliet, Traité de Zool. med. et agricole. (ed. 2, Paris.) xv + 737-1303 pp., figs. 495-892.

Gros, C.: 1849.—Fragments d'Helminthologie et de Physiologie microscopique. Bull. Soc. Imp. de Nat. de Moscow, 22: 549-573.

Hegner, R. W. 1925.—*Trichomonas vaginalis* Donne. Am. Jour. Hyg., 5: 302-308, 3 figs. in text.

Hegner, R. W. and Ratcliffe, H. 1927.—Trichomonads from the mouth of the dog. Jour. Paras., 14: 51-53, 1 fig. in text.

Hinshaw, H. C. 1926a.—On the morphology and mitosis of *Trichomonas buccalis* (Goodey) Kofoid. Univ. Calif. Publ. Zool., 29: 159-174, pl. 11, 2 figs. in text.

1926b.—Correlation of protozoan infections of the human mouth with extent of certain lesions in Pyorrhea alveolaris. Proc. Soc. Exp. Biol. Med., 21: 71-73.

1927.—Cultivation of *Trichomonas buccalis*, a protozoan of the human mouth. Univ. Calif. Publ. Zool., 31: 31-51.

1928.—Experimental infection of dogs with *Endamoeba gingivalis* and *Trichomonas buccalis* of human mouth. Proc. Soc. Exp. Biol. Med., 25: 430-431.

Hinshaw, H. C. and Simonton, F. V. 1928.—The amoebas of the mouth in relation to incidence of pyorrhea, clinical symptoms, extent of lesions and age of patients. Jour. Dent. Res. Unpublished.

Hogue, M. J. 1926.—Studies on *Trichomonas buccalis*. Am. Jour. Trop. Med., 6: 75-88, 1 pl.

Howitt, B. F. 1925.—The culture of *Endamoeba gingivalis* (Gros.). Univ. Calif. Publ. Zool., 28: 65-126, pls. 10-12.

1926a.—The effect of certain drugs and dyes upon the growth of *Endamoeba gingivalis* (Gros) *in vitro*. Univ. Calif. Publ. Zool., 28: 173-182.

1926b.—Experiments with *Endamoeba gingivalis* (Gros) in mixed bacterial cultures; in filtered saliva; on a solid base; with peritoneal cells, and with digestive secretions. Univ. Calif. Publ. Zool., 28: 183-202, pls. 16-17.

Kartulis, S. 1893.—Ueber pathogene Protozoen bei dem Menschen. I. Gregarinose der Leber und bei Bauch Muskeln. II. Amoeben bei Knochennekrose (Osteomyelitis) des Unterkiefers. Ztschr. f. Hyg. u. Infekt., 13: 1-14, pls. 1-2.

Koch, D. A. 1926.—An experimental study of the effects of dyes, of dye mixtures, and of disinfectants upon *Endamoeba gingivalis* (Gros) *in vitro*. Univ. Calif. Publ. Zool., 29: 241-266.

1927.—Relation of moisture and temperature to the viability of *Endamoeba gingivalis* (Gros) *in vitro*. Univ Calif. Publ. Zool., 31: 17-29, 2 figs. in text.

Kofoid, C. A. 1920.—A critical review of the nomenclature of human intestinal flagellates, *Cercomonas*, *Chilomastix*, *Trichomonas*, *Tetratrichomonas* and *Giardia*. Univ. Calif. Publ. Zool., 20: 145-168, 9 figs. in text.

1928.—*Councilmania tenuis* and *C. dissimilis*, intestinal amebas of man. Arch. Int. Med., 41: 558-573, 5 figs. in text.

Kofoid, C. A. and Hinshaw, H. C. 1929.—Distribution *in situ* of *Endamoeba gingivalis* and its relation to the filamentous bacteria in calculus. In press.

Kofoid, C. A. and Swezy, O. 1924.—The cytology of *Endamoeba gingivalis* (Gros) Brumpt with special reference to the determination of the amoebae in bone marrow in arthritis deformans of Ely's second type. Univ. Calif. Publ. Zool., 26: 165-198, pls. 14-16, 1 fig. in text.

Lynch, K. M. 1915.—Trichomoniasis of the vagina and the mouth, cultivation of causative organism and experimental infection. Am. Jour. Trop. Dis. and Prevent. Med., 2: 627-634.

1922.—Cultivation of trichomonads and the differentiation of the flagellates. Jour. Am. Med. Ass., 79: 1130-1133, 1 fig. in text.

Mitchell, O. W. H., Culpepper, W. L. and Ayer, W. D. 1916.—*Endamoeba buccalis* in the mouths of institutional children. Jour. Med. Res., 35: N. S. 30: 51-53.

Müller, O. F. 1773-1774.—Vermium terrestrium et fluviatilium, seu animalium infusiorium, helminthocorum et testaceorum, non marinorum, succincta historia. (Havniae et Lipsiae, Heineck et Taber.) V. 1 (1) xxxiv + 135, V. 1 (2) 72 + viii, V. 2, xxxvi + 214 + x.

Naesland, C. 1925.—Studies of Actinomyces from the oral cavity. Acta. Path. Micro. Scand., 2: 110-140, 13 figs. in text.

Nowlin, N. 1917a.—*Endamoeba buccalis.* I. Its multiplication and periodicity. Jour. Paras., 3: 143-149, 1 fig. in text.

1917b.—*Endamoeba buccalis.* II. Its reactions and food taking. Jour. Paras., 4: 21-24, 2 figs. in text.

Ohira, T. and Noguchi, H. 1917.—The cultivation of *Trichomonas* from the human mouth. (*Tetratrichomonas hominis.*) Jour. Exp. Med., 25: 341-347, pls. 34-37.

Prowazek, S. von. 1904.—*Entamoeba buccalis* n. sp. vorläufige Mitteilungen. Arb. Kais. Gesund., 21: 42-44.

Schmidt, A. 1895.—Ueber parasitäre Protozoen. (*Trichomonas pulmonalis.*) München. Med. Wochenschr., 42: 1181-1182.

Smith, A. J. and Barrett, M. T. 1915.—The parasite of oral endamebiasis, *Endamoeba gingivalis* (Gros). Jour. Paras., 1: 159-175, 1 pl.

Smith, A. J. Middleton, W. S. and Barrett, M. T. 1914.—The tonsils as a habitat of oral endamebiasis. Jour. Am. Med. Ass., 63: 1746-1749.

Steinberg, S. 1862.—An investigation of the soft white matter which accumulates on the teeth and between them. Sevremennaya Meditsina, Kiev, 20: & 24: 377-452.

Tibaldi, E. 1920.—Sopra una nuova specie di ameba parasita trovato nelle tonsile. (*Entamoeba macrohyalina.*) Ann. de Igiene, 30: 613-621, pl. 1.

Verdun, P. and Bruyant, L. 1907.—Sur la présence d'amibes dans le pus d'abscés de la région malaire. C. R. Soc. Biol., Paris, 63: 161-163.

Wells, H. G. 1925.—Chemical pathology. (Philadelphia and London, Saunders.) 790 pp.

Wenyon, C. M. 1926.—Protozoology. A manual for medical men, veterinarians and zoologists. (New York, William Wood Co.) 2 vols., xvi + 1563 pp., 20 colored pls., 565 figs. in text.

Williams, A. W., von Sholly, A., Rosenberg, C., and Mann, A. G. 1914-1915.—Significance and prevention of amebic infections in the mouths of children. Collected Studies from the Bur. Lab., City of N. Y., 8: 405-408.

EIMERIA ELLIPSOIDALIS NOV.SPEC., A NEW COCCIDIUM OF CATTLE

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AND
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In a previous paper we reported the results of a survey of the protozoan population of the feces of calves. Up to the present writing forty calves have been examined. Three of these animals were found to be infected with coccidia of the genus Eimeria. The oocysts of two calves were so distinctly different from those of the third, as to suggest that we were dealing with two distinct species. The idea that there may be more than one species of coccidia parasitic in the cow is not new since Reichenow, Smith and Graybill, Yakimoff and Galouzo and others have previously submitted evidence in support of it.

The history of bovine coccidiosis begins with Zürn (1878) who wrote an account of his observations upon the intestine of a calf which had died of acute intestinal inflammation caused by coccidia; or, to quote directly, "durch Gregarinen hervorgerufene Enteritis in optima forma." No description of the parasite is given. He does mention, however, that rabbits were not kept with the calves, and thus implies that the rabbit coccidium was not responsible for the infection in the calf.

Rivolta (1878) gave the name *Cytospermium zürni* to the "gregarine" which Zürn had observed. He states that Zürn did not give a definite description of the parasite, and he gives none beyond saying that it seems identical to the species described by Eimer in rats or to the psorosperm of the rabbit. Although neither Zürn nor Rivolta gave a definite description of the parasite, both had in mind the causative agent of a particular form of enteritis of cattle.

Beginning with Guillebeau (1893) a number of workers reported two types of oocysts of Eimeria from cattle. (*Vide* Yakimoff and Galouzo, 1927, for adequate historical account). Smith and Graybill (1918) designated the two types which they observed in calves in New Jersey as two species. The one species was usually elliptical, occasionally ovoid or circular. Most of the individuals measured would fall within a range of length from 13.1 to 28.7μ and of width from 12.3 to 20.5μ; average length, 18.6μ; average width, 14.8μ. There was no residual body within either the cyst or the spore after development. The second species was distinctly ovoid and brownish or colorless. There was no residual body within the cyst, but one was present in each spore. Size of oocysts, 25.8 to 41.8μ by 16.4 to 24.6μ; average, 29.9 by 19.9μ.

Yakimoff and Galouzo (1927) in their recent extensive survey of coccidiosis of cattle in Russia noted likewise oocysts of two species which they designate as the round and the egg-shaped. The former has an average size of 17.1 by 17.1μ, has no residual body in cyst or spore. For this species these authors retain the name *Eimeria zürni*. The second has an average size of 31.5 by 21.6μ, and has a residual body in each spore. This species they call *Eimeria smithi* in honor of Theobald Smith.

The oocysts of the species we encountered in two of our calves were of the ovoid or egg-shaped type, broader at one end than at the other (Fig. 1). The cyst wall is considerably thinner at the narrower end than at the broader. There is a salmon tint to the cyst. Measurements gave a length of from 25 to 32μ and width of 17 to 22μ; average size, 29.7 by 20.2μ. These average measurements are very near those given by Smith and Graybill, and not very far from those given by Yakimoff and Galouzo. The ratio of average length to average width was 1.47. When these washed oocysts were kept in 1% potassium dichromate solution for about two weeks they developed four spores, each spore with a protoplasmic residium or residual body. This species agrees in every respect with *Eimeria smithi* Yakimoff and Galouzo 1927.

The oocysts of the second species, encountered only in one calf, were predominantly ellipsoidal. Ovoid or approximately round ones were rarely seen. Although moderately numerous in our smears these oocysts were almost overlooked at first because they were so inconspicuous and colorless. We debated whether they might not be the spores of a fungus. In the fresh oocysts the protoplasm almost completely filled the cyst. After about four days the protoplasmic mass contracted and became a compact sphere (Fig. 2). The fecal matter containing the oocysts was diluted with 1% potassium dichromate, kept at room temperature, and observed at intervals. In about two weeks four sporoblasts were found in each oocyst (Fig. 3). Four days later each sporoblast had developed into a spore containing two sporozoites and a residual mass (Fig. 4). There was no residual body in the cyst. The nuclei of the sporozoites were not visible to us. Measurements gave a length of from 20 to 26μ and a width of from 13 to 17μ; average size, 23.4 by 15.9μ; ratio of average length to average width, 1.47.

This species has the following characters in common with *E. smithi:* (1) There is no residual body in the mature cyst. (2) There is a residual body in the spore. (3) The ratio of average length to average width in both is 1.47. It differs from *E. smithi* in these characters: (1) It is colorless and more inconspicuous. (2) The oocyst wall is thinner, but does not thin out at the micropyle end proportionately to that of *E. smithi*. (3) It is ellipsoidal in shape. (4) It is considerably smaller in size.

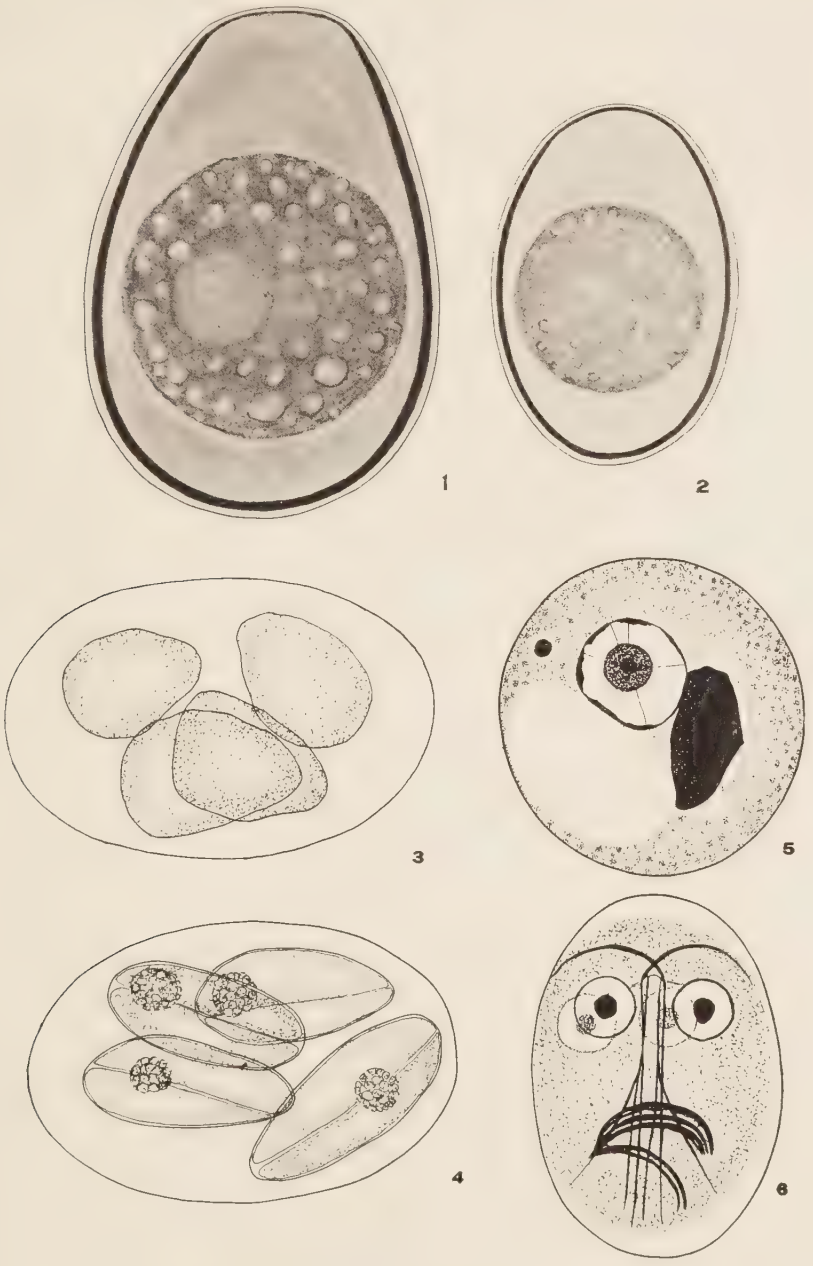

PLATE X

EXPLANATION OF PLATE X

Fig. 1.—Oocyst of *Eimeria smithi*. × ca 2,120.

Fig. 2.—Oocyst of *Eimeria ellipsoidalis* four days after collecting. × ca 2,120.

Fig. 3.—Oocyst of *E. ellipsoidalis* with four sporoblasts. × ca 2,240.

Fig. 4.—Same with each sporoblast containing two sporozoites and a residual body. × ca 2,240.

Fig. 5.—Cyst of *Endamoeba bovis*. × ca 3,405.

Fig. 6.—Cyst of *Giardia bovis*. × ca 3,405.

We have never seen *E. zürni,* but from the accounts of Jowett, Smith and Graybill, Yakimoff and Galouzo and others this form does not have a residual body inside the spore. Its tendency toward the spherical shape is another difference.

Most previous authors have observed mixed infections of *E. zürni* and *E. smithi* in the animals they studied. Smith and Graybill, however, reported a pure infection of their elliptical species, and Yakimoff and Galouzo observed some pure infections of each of the two species they studied. Our three animals had pure infections.

The caretaker of these three calves, which were from the Iowa State College dairy farm, stated that to his knowledge none of them had ever suffered diarrhea or dysentery. This suggests that these two species may not be so pathogenic as is *E. zürni,* which seems usually to be present in "rote Ruhr," or coccidian dysentery of cattle as Galli-Valerio prefers to call it. The infections we observed were in calves of from two to six months of age, but were not to be considered heavy infections if one may judge from the number of oocysts. It remains for future investigators to determine whether clinical symptoms are ever produced by either *E. smithi* or *E. ellipsoidalis.* A reading of the literature indicates that in cattle *E. zürni* is preeminently the pathogenic coccidium. Our experience indicates that the other species may not be sufficiently injurious to produce clinical symptoms in the host.

We have included in the plate figures of the cysts of *Endamoeba bovis* Liebetanz 1905 and *Giardia bovis* Fantham 1921, as drawn from iron-hematoxylin stained slides.

Literature Cited

Becker, E. R. and Frye, W. W. 1928.—Some Protozoa found in the Feces of Cattle, Proc. Ia. Acad. Sci. (In press.)

Galli-Valerio, B. 1918.—Coccidia in the Intestines—Red Dysentery of Cattle—Observations on *Eimeria zürni Rivolta.* (A translation.) Vet. Jour. 74: 219-223.

Guillebeau, A. 1893.—Ueber das Vorkommen von *Coccidium oviforme* bei der roten Ruhr des Rindes. Centralblatt f. Bakt. u. Parasitenkunde, 14: 467-468.

Reichenow, E. 1921.—Die Coccidien. Handbuch der Pathogenen Protozoen by Prowazek and Nöller, pp. 1136-1272.

Rivolta, S. 1878.—Della gregarinosi dei polli e dell'ordinamento delle gregarine e dei prorospermi degli animali domestici. Giorn. Anat. Fisiol. Pat. Anim., Pisa, 10: 220.

Smith, T. and Graybill, H. W. 1918. Coccidiosis in young calves. Jour. Exp. Med., 28: 89-108.

Yakimoff, W. L., and Galouzo, J. G. 1927.—Zur Frage über Rindercoccidien. Arc. Prot., 58: 185-200.

Zürn, F. A. 1878.—Die kugel- und eiformigen Psorospermium als Ursache von Krankheiten bei Haustieren. Vortrage Thier-Ärzte 1, No. 2.

THE ASEXUAL CYCLE IN *LEUCOCYTOZOON ANATIS**

ERNEST HARTMAN

Young ducks were brought to the University of Michigan Biological Station in the summer of 1927. These were not infected upon arrival but plainly showed *Leucocytozoon anatis* after being kept ten days near other ducks infected with this parasite. During this time blood samples were taken daily. Since the manner and time of infection were not determined, it was not possible to be accurate in giving the age of any parasite subsequently found. However, from the daily samples it was found possible to work out the various developmental stages and to assign ages which appear correct to within not more than three days.

The earliest parasites found were in young red blood cells (Figs. 3-5), and appeared similar to young stages of bird malaria. The parasite possesses a cytoplasm which with tetrachrome stain distinctly stains blue, and a nucleus which stains red. The normal young red blood corpuscle has a larger and less compact nucleus than the older cell; the cytoplasm takes more azur stain and less eosin than do the mature red cells. Corpuscles can be found which show a series of gradations from the early to the later stages. The parasitized corpuscles do not go through these normal maturing stages but change in other ways as pointed out later. At first the nucleus of the parasitized cell enlarges, the cell membrane enlarges, becoming more spherical and later the parasite comes to be partly surrounded by the nucleus. This is the beginning of the elongation of the nucleus of the host cell which is so readily recognized in certain Leucocytozoa. Thus in some cases development seems to proceed regularly from small to large forms, in sequence figures 3, 4, 5, 6, 7 10 and 11. In other cases the nucleus seems to become scattered into chromidia and the protoplasm of the parasite surrounds the host-cell nucleus (Fig. 9) so that the whole appears similar to a large mononuclear leucocyte. The parasite grows to approximately full size with the host cell and parasite both being spheroid and the nucleus partly surrounding the parasite. Up to this stage the host cell nucleus is not compact as it is later, but the chromatin is in somewhat localized masses with nearly clear spaces between. By the time the parasite has attained full size, the macrogametocytes can be readily distinguished. From the numbers present and from the appearance of division which occurs in some, those which are developing into microgametocytes and those which are developing into schizonts seem indistinguishable as both take very little azur and in both the nucleus stains well with eosin.

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* Contribution No. 333 from the Department of Zoology of the University of Illinois, and from the University of Michigan Biological Station.

One example of merozoites was found. They were liberated in the making of the blood film and although not all were completely spread out, there appeared to be about 70 merozoites. Since thousands of parasites were observed, and this was the only case of merozoites found, the chance of two schizonts being so close together would be only one in perhaps several billion. The individual merozoites were about one micron in diameter and distinctly showed an azur staining cytoplasm and a nucleus which did not in any way resemble the chromatin of bacteria. These merozoites appear very similar to those of Plasmodium, but are a little smaller than most related merozoites.

The earliest recognizable gametocytes are in cells which are very weak-walled and spheroid. This stage of development is reached in about eight days. In about two days more the cell has elongated so that the nucleus of the host cell is longer than the parasite which has become somewhat cylindrical in shape. The wall of the host cell has drawn out so that there are two very pointed ends which gradually taper back, being in the center jurst large enough to enclose the parasite and the host cell nucleus. At first these ends are fragile, but later become more rigid. There is a space beyond the nucleus and parasite which is apparently filled with a thin, watery fluid.

The genus Leucocytozoon is one which has been described as not forming pigment. The gametocytes of *Leucocytozoon anatis* definitely contain pigment in very small granules scattered throughout the cytoplasm. This very fine pigment shows in the photograph (Fig. 1), as small light areas due to the refraction of the light by the pigment. In some parasites this pigment is not distinguishable, while in others it is very plain. The macrogametocytes contain more pigment than do the microgametocytes. Apparently the schizont does not form pigment.

During the time since Danilewsky (1890) found parasites which he called Leucocytozoaires, several workers have previously reported what they considered to be the asexual cycle in the genus Leucocytozoon. Fantham (1910), working with the Leucocytozoa of the grouse, reported what he considered to be schizogony stages. It is difficult to determine from the figures whether any similar stages have been found in the present study. The figures which he considers to be the dividing forms bear a superficial resemblance to Coccidia. He expresses the opinion that all Leucocytozoa need not have the same schizogony method. In the light of the present study, it must be considered that Fantham did not have the dividing forms. Moldovan (1914) also reports the schizogony cycle. In the present study, large cells which appear similar to the figures drawn by Moldovan have been found. The first conclusion was, after the manner of Moldovan, that the schizonts were being viewed but subsequent observations disproved this even before the markedly different merozoites were found. Under the microscope

these forms appear much more similar to leucocytes which have ingested and partly destroyed bacteria, than they appear like a normal protozoa in an early sporozoite stage. These forms are found near the height of the gametocyte number and are not followed by a marked increase in either trophozoites or gametocytes.

Knuth and Magdeburg (1922), describe the schizogony cycle in *Leucocytozoon anseris*. The figures they have drawn most nearly resemble the early developmental stages of the gametocyte before the ends of the cell have become pointed. Coles (1914) has correctly recognized the schizont of the genus Leucocytozoon. His photographs very clearly show the usual characteristics of the merozoites and are quite similar to the ones photographed in the present study. He failed to find the very youngest stages and thus complete the cycle, so that his article, by some, might be considered unconvincing. Wenyon (1909), tentatively put forth the claim that the parasite enters red blood cells rather than leucocytes, as the name of the genus would indicate. He was not successful in finding quite such a young stage as photographed in figure 2. Thus while the cells seem to be similar to a red cell, he was cautious in his statement that it was the red cell. In the present case there can be no doubt that the young parasites do enter red cells and gradually alter the appearance of the cell.

The present study confirms in part both Coles and Wenyon. Both of these workers made correct observations but were unsuccessful in getting the complete story. So far as known to the present author, this is the first record of the complete asexual life cycle of a Leucocytozoon, although it must be admitted that here there is a slight piecing together of the stages, in that not all were taken at succeeding times from the same individual host. However, several forms could be found, although specimens properly stained for photographing were not found. The very earliest forms were seen rather infrequently, for which there seems to be no obvious explanation.

From experimental evidence it seems possible that the parasite does not readily multiply in the host, although it does, in common with other Hemosporidia, maintain a low grade infection for a long time. One duck was observed to maintain an infection fifteen months, another twelve, and another ten months. In two of these a slight relapse, as measured by the appearance of gametocytes occurred, but it was not so severe as the initial infections. Wenyon (1909), says: "It is very probable that the young birds become infected soon after hatching, and that the infection gradually gets smaller with the increase in age." This is probably usually true, insofar as the young birds becoming infected is concerned; but the infection is to be regarded as one with rising and falling severity, just as in the other observed members of the family. Also in different hosts considerable variations have been observed in the height to which the infection goes before it causes death or naturally

subsides. The observed cases showed a higher initial infection than did the observed relapses. However, it is possible that the relapse may in some cases be more severe than the initial infection similar to what is known to occur in malaria.

In the experimental work uninfected ducks (by examination), were brought to the University of Michigan Biological Station and after one hour were placed in tight cages screened with wire cloth having twenty meshes per inch. Since the parasite occurs naturally in this location, and other ducks not protected had a few days previously become infected, and were then but a short distance away, it is very probable that during this one hour part of the ducks became infected as seemed to be indicated by later developments. After three days six were placed outside and near the other ducks which had already the Leucocytozoa. Five of these which lived, showed large gametocytes with pointed ends of the host cell at the end of about ten days. Of thirteen which were kept in the cages continuously, nine remained uninfected and four showed parasites in about the same numbers as was shown in those which had been outside. It seems most likely that these parasites had developed from an initial infection received during the first hour after arrival when they were unprotected. Since there seemed to be no point in keeping the infected ducks protected, all were placed outside together and blood samples were taken daily as had been done with those which were outside. It was a surprise to find that the infections in the four which had been kept inside did not parallel the infection of the others, but showed only a few mature gametocytes until about two weeks later. Although this point needs more study, it would seem from the evidence at hand that the initial infection produces some gametocytes and a very few merozoites, and that in case the host is subjected to heavy and repeated infection, a very large number of gametocytes may be found. A few parasites do not seem to make the duck immune to another infection, but a heavy infection seems to cause an immunity which results in the disappearance of the larger number of parasites similar to what occurs in bird and in human malaria.

References Cited

Coles, A. C. 1914.—Blood parasites found in mammals, birds, and fishes in England. Parasit., 7: 17-61.

Danilewsky, V. 1890.—Développement des parasites malariques dans les leucocytes des oiseaux (*Leucocytoaires*). Ann. Inst. Pasteur, 4: 427-431.

Fantham, H. B. 1910.—Observations on the Parasitic Protozoa of the Red Grouse (*Lagopus scoticus*), with a note on the Grouse Fly. Proc. Zool. Soc. of London, 2: 692-708.

Knuth, P. and Magdeburg, F. 1922.—Uber ein durch Leukozytozoen verursachtes Sterben junger Gänse. Berliner Tierarztliche Wochenschrift, 38: 359-361.

Moldovon, J. 1914.—Untersuchungen über den Zeugungskreis des *Leucocytozoon ziemanni* (Laveran).

Wenyon, C. M. 1909.—Report of traveling pathologist and protozoologist. Third Report, Wellcome Trop. Res. Lab., Khartoum, 121-168.

Explanation of Plate XI

Microphotographs of *Leucocytozoon anatis*. Magnification, 2,000 diameters.

Fig. 1.—Microgametocyte and macrogametocyte, showing the pointed ends of the host cell in approximately normal living position; the elongated host cell nucleus which in the microgametocyte is partly coiled around the parasite; and the pigment granules in the gametocyte.

Fig. 2.—Merozoites as they appeared on the slide, having been liberated as the film was made.

Figs. 3 and 4.—Young parasites in red blood cells. The parasite nucleus appears dark with a small area of cytoplasm surrounding it. Probably about one to two days old.

Figs. 5 and 6.—Later stages in development, showing the beginning of encroachment on the nucleus of the host cell. Probably about three or four days' development.

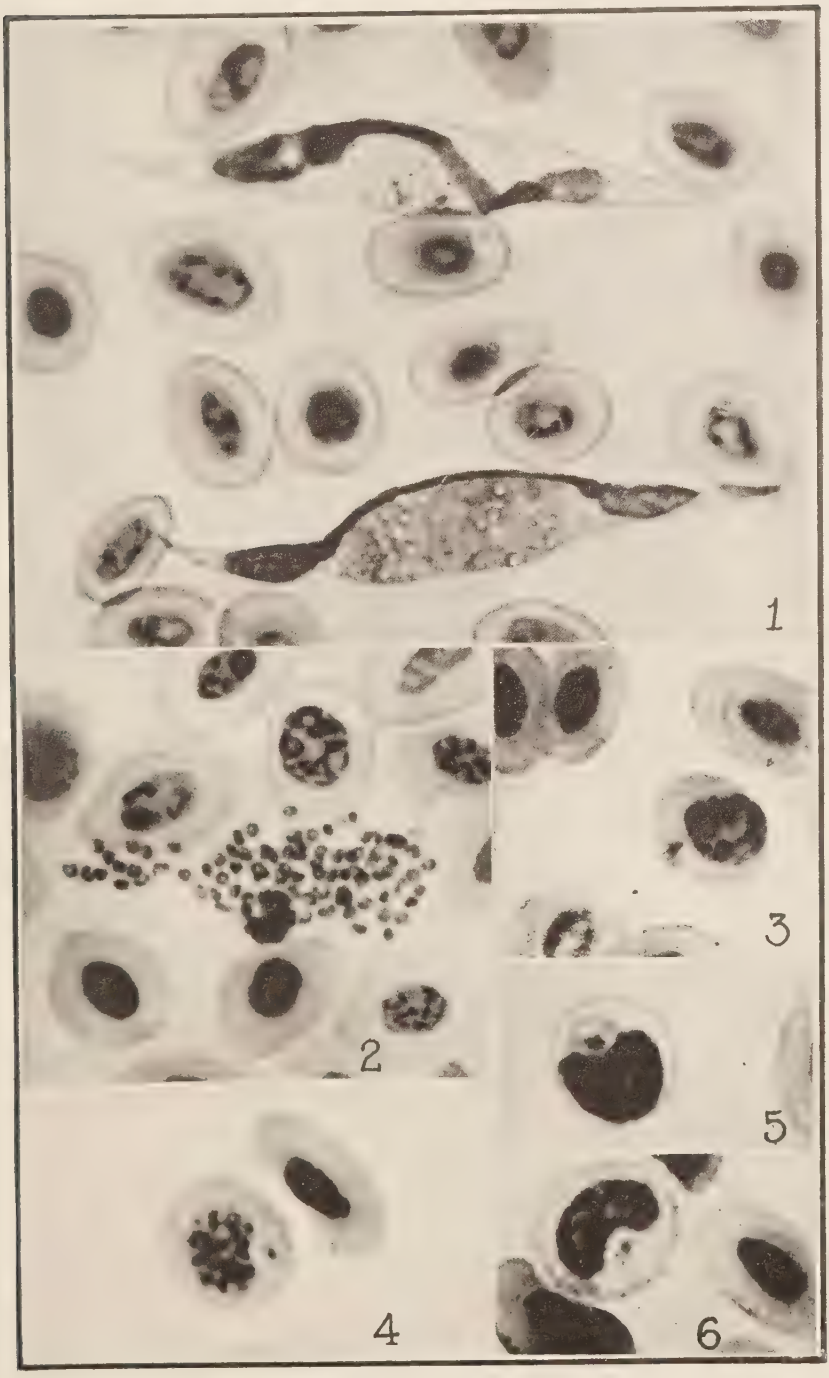

PLATE XI

EXPLANATION OF PLATE XII

Fig. 7.—Later stage showing further enlargement of the parasite host cell and host cell nucleus. About five days' development.

Fig. 8.—Later stage in which the host cell has enlarged more rapidly than the parasite and in this particular case the nucleus of the host cell is almost divided.

Fig. 9.—Two parasites which appear similar to large mononuclear leukocytes, but which have no counterpart in normal duck blood, and which are followed one and two days later by similar forms more enlarged and plainly Leucocytozoon.

Fig. 10.—Practically full grown macrogametocyte, showing the membrane of the host cell larger than sufficient to enclose the nucleus and the parasite.

Fig. 11.—Microgametocytes similar to the preceding but in which the fragile host cell membranes have ruptured, liberating the parasites and leaving the host cell nuclei near and plainly visible.

The latter two stages are always reached in *Leucocytozoon anatis* before the host cell attains the pointed ends. In this stage the host cell membrane is very easily broken, in contrast to the comparatively rigid and constant host cell form shown in figure 1. It is suggested that such forms may be the kinds which have been used in certain descriptions of new species of Leucocytozoon.

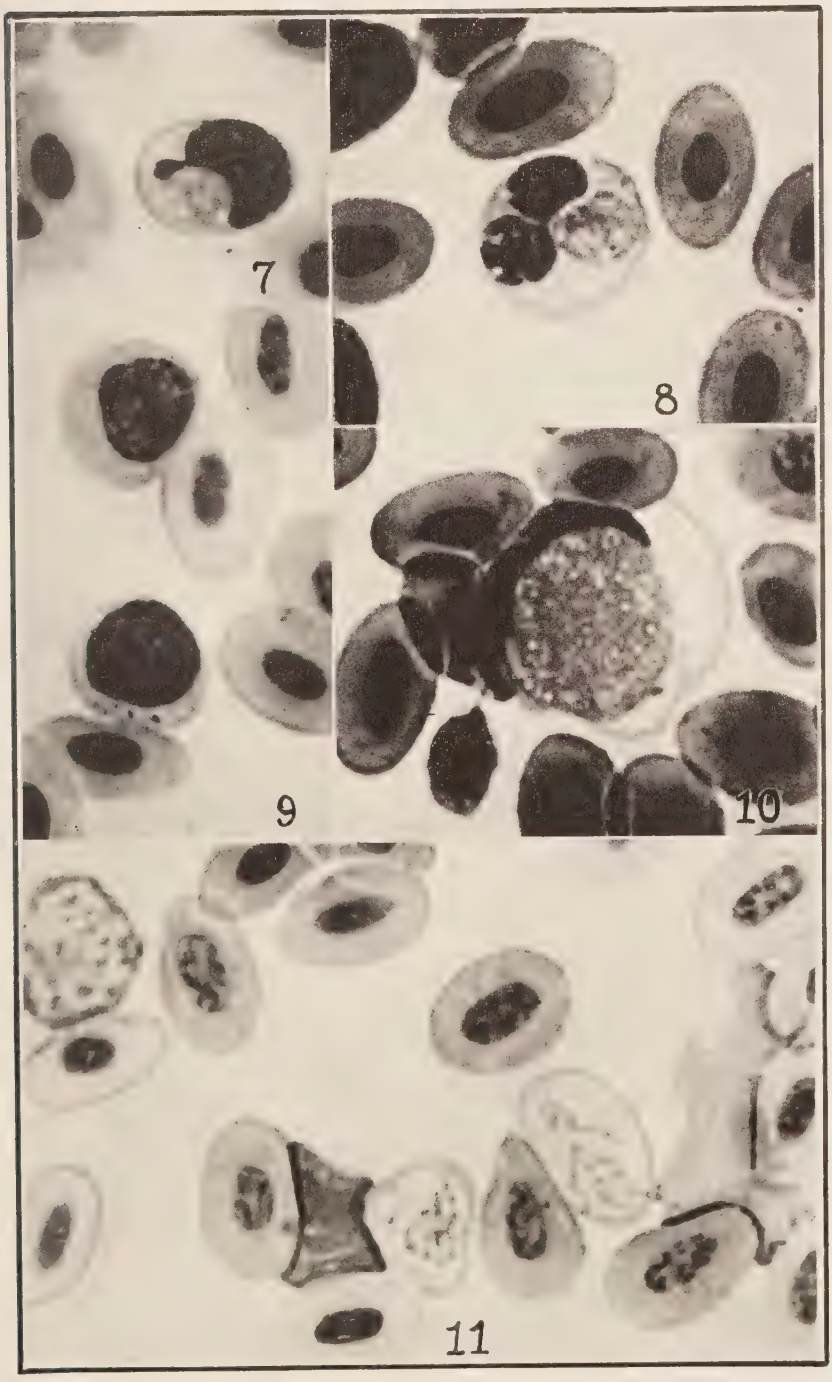

PLATE XII

NOTE ON A NEW SPECIES OF COCCIDIA FROM THE POCKET GOPHER (*GEOMYS BURSARIUS*) (SHAW)*

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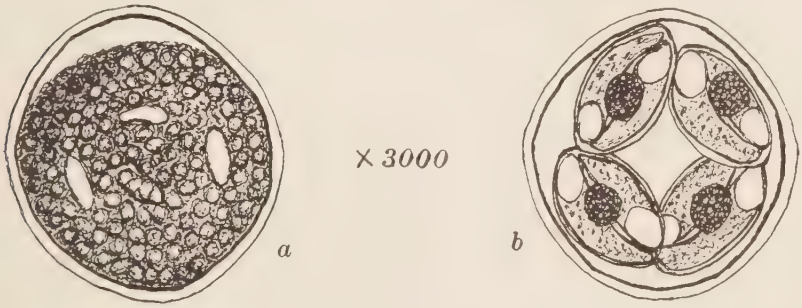
University of Nebraska

In April, 1928, the author caught a young pocket gopher in Lincoln, Nebraska. It measured five inches from tip of nose to root of tail. Coccidia were found to be quite numerous in the small and large intestine. There was no evidence of blood mixed with the intestinal contents, nor any apparent macroscopic damage to the intestinal tract. The intestinal contents were mixed with a 2% potassium dichromate solution, strained and kept at room temperature to observe further development



Text-fig.—*a*, Oöcyst of *Eimeria geomydis* from the intestine, × 3,000; *b*, mature oöcyst, × 3,000.

of the coccidia. Development proved to be characteristic of the genus Eimeria. According to Muyori (1926) the mature oöcyst contained four sporocysts, each with two sporozoites. The writer has not found any record of coccidia in the pocket gopher (*Geomys bursarius*) (Shaw), and suggests the name *Eimeria geomydis* n. sp.

Only the unsegmented stage was found in the intestinal tract (Fig. *a*). No schizogenic stages, nor any forms of coccidia were found in the sectioned and stained liver tissue. The oöcysts are transparent, colorless, slightly oval to spherical and do not contain a residual body. The cytoplasmic material almost entirely fills the oöcyst. The wall is double layered, regular in outline, colorless and 0.5μ thick. A micropyle

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was observed only a few times in a large number of coccidia examined. In four days oöcysts were found to contain four sporocysts, each containing two sporozoites and a residual body.

Thirty-five mature oöcysts varied in length from 11.6 to 14.9μ and in width 11.6 to 13.3μ. The average length was 13.3μ and the average width 12.5μ. The average form index, 0.9375. The sporocysts measured 5 to 6.6μ in length and 4.2 to 5μ in width. Each sporocyst contained two sporozoites 4 to 7μ in length and 1.5 to 2.5μ in width. A nucleus could not be seen in the sporozoites. A rounded residue body composed of highly refractive granules was found in each sporocyst (Fig. *b*).

Reference Cited

Wenyon, C. M. 1926.—Protozoology. Vol. II, pp. 779-869. London.

NEW CARYOPHYLLAEIDAE FROM NORTH AMERICA*

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During the completion of a monograph on the Caryophyllaeidae of North America which is now in press the author encountered several interesting new species which should be brought to the attention of those interested in this group. It is of course necessary to straighten out the classification before it is possible to contemplate upon phylogenetic relationships or to delve into life history studies. In a previous paper (Hunter, 1927) the author summarized the earlier contributions and presented a summary of his proposed revision of the family Caryophyllaeidae. This included the erection of new subfamilies, the redescription of four species previously found on this continent and the addition of three new genera and four new species.

Other workers have recently added to the literature since the contributions to the knowledge of the Caryophyllaeidae by Nybelin (1922), Woodland (1923, 1924, 1926) and Fuhrmann and Baer (1925). Bovien (1926) briefly describes six new species which he tentatively places in the genus Caryophyllaeus and one new genus and species, *Djombangia penetrans*. Further study of the six species will undoubtedly bring about a reclassification of the parasites as many show characters which will place them in the Lytocestinae. Motomura follows in 1927 with a new species, *Caryophyllaeus gotoi*. Last year Baylis (1928) added a new genus and species *Lytocestoïdes tanganyikae* while Wisniewski described a new parasite, *Archigetes cryptobothrius* from the Tubificid worm, *Limnodrilus Hoffmeisteri*. The present paper adds the following from North America:

Pseudolytocestinae n. subfam.
Pseudolytocestus differtus n. g., n. sp.
Spartoides wardi n. g., n. sp.
Glaridacris confusus n. sp.
Biacetabulum meridianum n. sp.
Biacetabulum giganteum n. sp.

The author wishes to thank Dr. Parke H. Simer, Illinois Wesleyan University and Dr. Fred J. Holl, University of Buffalo for material from Mississippi and North Carolina, respectively, which furnishes the basis for the description of new species.

The new subfamily, Pseudolytocestinae, as the name implies possesses some of the characteristics of the Lytocestinae. The main difference

* Contribution from the Biological Laboratories of the Rensselaer Polytechnic Institute, No. 5.

lies in the position of the vitellaria in relation to the inner longitudinal muscles. In the former they arise in the medullary parenchyma, but extend for one third to one half their length into the cortical parenchyma while in the latter the vitellaria are *entirely* cortical. In the former, due to the medullary origin of the vitellaria and their cortical distribution, these glands are very irregular in shape; Capingens, for example, possesses dumb-bell shaped vitellaria. Likewise the hosts are entirely different, for the Pseudolytocestinae are confined to the Catostomidae while the Lytocestinae appear only in the Siluridae. The author had not realized the existence of the former group until several genera were found with the same arrangement of the vitellaria. Consequently in a previous paper (Hunter, 1927) *Capingens singularis,* although having the characteristics of the Pseudolytocestinae, was placed in the Lytocestinae. In this genus the vitellaria had a medullary origin and yet nearly one half of their length lay in the cortical parenchyma past the inner longitudinal muscles. The parasite was likewise found in members of the Catostomidae. It is therefore removed from the Lytocestinae and taken as the type genus of the Pseudolytocestinae.

The Lytocestinae remains then with but two genera, Lytocestus and Monobothroides. It seems that the genus Djombangia Bovien (1926) belongs in this subfamily as the vitellaria are figured entirely external to the inner muscle layer, the sexual apertures occur in the last fourth of the body and the uterine glands or "Radialzellen" of Will (1893) are present for a portion of the course of the uterus. Likewise it is probable that further study of the six other new species described by the same author will lead to their being placed in other genera and subfamilies. As noted by Baylis (1928) his new genus Lytocestoïdes probably belongs in the Lytocestinae even though the material was in a poor state of preservation and the details of the musculature could not be made out. Baylis mentions the proximity of the vitellaria to the body surface as well as the location of the excretory canals in relation to the vitellaria as reasons for placing this in the Lytocestinae. Another indication that this form was rightfully placed lies in the regular shape of the vitellaria, which is one of the distinguishing characteristics of the Lytocestinae when comparing it with the Pseudolytocestinae. In a previous paper (Hunter, 1927) the authority of Woodland (1926) was accepted for placing *Balanotaenia bancrofti* provisionally in the genus Lytocestus. Since then the author has had an opportunity to study Johnston's (1924) original description of this form which is apparently a valid genus. According to the emended description of this group, the genus is provisionally placed in the Lytocestinae. Woodland (1926) sensed this when he placed the form in his genus Lytocestus whose broad characters are taken over in part to form the subfamily

Lytocestinae. A full discussion of this appears in the monograph which is in press.

The emended description of the Lytocestinae will be given as well as that of the new subfamily Pseudolytocestinae in order to insure a complete understanding of the diagnostic characteristics. A summary of the family Caryophyllaeidae includes the following:

CARYOPHYLLAEIDAE Leuckart 1878

Caryophyllaeinae (Nybelin 1922)
- Caryophyllaeus Müller 1787 (type genus)
- Monobothrium Diesing 1863
- Glaridacris Cooper 1920
- Caryophyllaeides Nybelin 1922
- Biacetabulum Hunter 1927
- Hypocaryophyllaeus Hunter 1927

Pseudolytocestinae n. subfam.
- Capingens Hunter 1927 (type genus)
- Pseudolytocestus, n. g.
- Spartoides n. g

Lytocestinae Hunter 1927
- Lytocestus Cohn 1908 (type genus)
- Balanotaenia Johnston 1924
- Monobothroides Fuhrmann and Baer 1925
- Djombangia Bovien 1926
- Lytocestoïdes Baylis 1928

Wenyoninae Hunter 1927
- Wenyonia Woodland 1923 (type genus)

Lytocestinae Hunter, 1927, char. emend.

Subfamily diagnosis: Caryophyllaeidae with sexual apertures and ovary situated in last quarter of body. Inner longitudinal muscles *entirely* internal to regularly shaped vitellaria which are annularly arranged about muscles in cortical parenchyma. Uterine glands present. Parasitic in the Siluridae.

Type genus: Lytocestus Cohn, 1908.

Pseudolytocestinae, n. subfam.

Subfamily diagnosis: Caryophyllaeidae with sexual apertures and ovary situated in last fifth of body. Inner longitudinal muscles *partially* internal to irregularly shaped vitellaria which arise in medullary parenchyma and extend for one third to one half their length past the muscles into the cortical parenchyma where they are typically annularly arranged. Uterine glands present. Parasitic in the Catostomidae.

Type genus: Capingens Hunter, 1927.

Pseudolytocestus n. g.

Generic diagnosis: Pseudolytocestinae possessing little specialization of scolex. Cirrus opens separately on ventral surface or into shallow eversible atrium. Ovary "H" shaped and almost entirely medullary, only one third of ovarian follicles extend into cortical parenchyma. Uterine coils never extend anteriorly to cirrus sac with a maximum length one third that of testicular field. Post-ovarian vitellaria absent. Parasitic in intestine of Catostomidae. Development unknown.

Type and only species: *Pseudolytocestus differtus* n. sp.

Pseudolytocestus differtus n. sp. [Figs. 4-6]

Specific diagnosis: With characters of genus. Adult parasites free in intestine; length 10 to 20 mm., width 0.6 to 1.7 mm. Neck distinct and short, 0.9 to 1.7 mm. long and 0.6 to 0.95 mm. wide. No specialization of scolex apparent. Cuticula tripartite, composite measuring 8 to 10μ in thickness; subcuticula 15 to 20μ deep; cortical layer of parenchyma 68 to 81μ in depth. Cuticular, outer and inner longitudinal muscles present and prominent. Testes roughly oval, having a maximum diameter of 0.13 to 0.2 mm. and numbering between 725 and 775. Cirrus sac and external seminal vesicle form Greek letter "Λ", where they meet dorsally; cirrus sac forms an angle of less than 30 degrees with the vertical and fills entire medullary parenchyma dorso-ventrally being 0.2 to 0.5 mm. long. Circular muscles of this organ are weak. Female genital atrium opens on ventral surface 0.27 to 0.3 mm. posterior to male; utero-vaginal canal 0.16 to 0.34 mm. long, extending dorsally; uterus empties at middle of body from right. Vagina proper extends dorsally until 0.4 to 0.6 mm. from ventral surface but swings ventrally immediately. No receptaculum seminis present. Ovarian wings 0.6 to 1.3 mm. long by 0.2 to 0.4 mm. wide; these extend out past the inner longitudinal muscles in same manner as vitellaria; ovarian commissure entirely medullary, 0.23 to 0.27 mm. in diameter. Vitellaria measure 0.13 to 0.2 mm. in maximum diameter, are irregular in shape, extending past the inner longitudinal muscles and into cortical parenchyma. Eggs are large, ovoid and measure between 58 and 65μ in length and 35 to 40μ in breadth.

Host: *Ictiobus bubalus,* Tallahatchie River, Mississippi. In intestine.

Spartoides n. g.

Generic diagnosis: Pseudolytocestinae possessing three pairs of loculi on distinct scolex. Male and female pores open separately on ventral surface nearer posterior tip of body than anterior margin of last fourth of body length. Cirrus sac opens within the confines of ovarian wings. One row of main inner longitudinal muscles. Vitellaria and ovarian follicles are in part cortical to inner longitudinal muscles; ovarian commissure entirely medullary. Ovary "U" shaped, uterine coils extending anteriorly to cirrus sac for more than twice length of ovarian wings. Post-ovarian vitellaria absent. Parasitic in the Catostomidae.

Type and only species: *Spartoides wardi* n. sp.

Spartoides wardi n. sp. [Figs. 7-9]

Specific diagnosis: With characters of genus. Adult parasites 8 to 30 mm. in length and 0.3 to 0.7 mm. in width. Neck is distinct and long, ranging from 1.6 to 4.7 mm. in length and 0.2 to 0.5 mm. in

breadth. Maximum body width exclusive of scolex 0.5 mm.; cuticula 2 to 3μ in thickness; subcuticula and cortical parenchyma fused measuring 42 to 58μ deep. Inner longitudinal muscles present and prominent outer longitudinal muscles absent. Testes numbering between 65 and 105 having a maximum diameter of 0.13 to 0.2 mm. Cirrus sac oval to triangular, occupying nearly all of medullary parenchyma; maximum diameter 0.09 to 0.14 mm.; circular muscles thin 9 to 12μ in width. Seminal vesicle prominent measuring about 0.13 mm. in length; circular muscles of this organ are 30 to 45μ thick. Male and female reproductive systems open on surface 60 to 85μ apart. Vagina very short and straight, not forming a receptaculum seminis, but extends dorsally to join combined ovi- and vitelline ducts immediately. Vitellaria arise in medullary parenchyma but extend past the inner longitudinal muscles for about one third to one half their length. Six pairs of main excretory canals present terminating in typical excretory vesicle. Eggs ovoid measuring 42 to 54μ in length and 22 to 27μ in width.

Host: *Carpiodes carpio,* Rock and Mississippi Rivers, Illinois and Iowa; *Carpiodes thompsoni,* Lake Pepin (Mississippi River), Minnesota; *Ictiobus cyprinella,* Rock River and Lake Pepin, Minnesota. In intestine.

The following new species belong in the Caryophyllaeinae.

Glaridacris confusus n. sp. [Figs. 1-3]

Specific diagnosis: With characters of genus. Adults usually 3 to 7 mm. in length and 0.2 to 0.8 mm. in breadth. Scolex oval at base, tapering distally to chisel shaped extremity cut by six loculi usually topped by a terminal disc. Inner longitudinal muscles are drawn into four prominent and four weaker groups of fasiculi which are confined to lateral thirds of base of scolex; outer longitudinal muscles rudimentary. Cuticula 2 to 4μ in thickness; subcuticula and cortical parenchyma fused having combined width of 15 to 40μ except in neck and scolex. Medullary parenchyma occupies three forths of body width. Testes large measuring 0.1 to 0.3 mm. in length by 0.1 to 0.13 mm. in breadth and number 25 to 35, occurring in two parallel rows. Cirrus sac fills one third to one half of medullary parenchyma and has a maximum diameter of 0.16 mm.; circular muscles are 10 to 17μ in thickness. Male and female reproductive systems open on surface 20 to 55μ apart. Vagina forms a large receptaculum seminis which is "S" shaped in dorso-ventral plane and situated anterior and dorsal to ovarian commissure; it measures 0.13 to 0.27 mm. in length and 0.031 to 0.067 mm. in width. Vitellaria are confined to two lateral rows and measure 0.067 to 0.135 mm. in maximum diameter. Eggs are small, ovoid and measure 37 to 48μ in length and 20 to 31μ in width.

Host: *Ictiobus bulbalus,* Rock River, Illinois, Mississippi River, Iowa, and Tallahatchie River, Mississippi; Ictiobus sp., and *Dorosoma cepedianum,* Tallahatchie River, Mississippi.

Biacetabulum meridianum n. sp. [Figs. 14-17]

Specific diagnosis: With characters of genus. Adult parasites measuring 5 to 15 mm. in length and 0.4 to 0.8 mm. in width. Scolex fairly prominent, is broader at the base than at the distal extremity and is set off from body by well defined neck. Cuticula 4 to 6μ in thickness; subcuticula 6 to 16μ deep, followed by cortical parenchyma measuring 50 to 70μ in thickness. Cuticular, outer and inner longitudinal muscles present and prominent. Testes numbering 65 to 95 are roughly oval and possess a maximum diameter varying between 0.13 and 0.15 mm. Vas deferens confined medianly between uterine coils; ductus ejaculatorius short; seminal vesicle long and "L" shaped, measuring 0.13 to 0.27 mm. in greatest length. Cirrus sac round, occupying one fourth of medullary parenchyma with a diameter varying between 0.13 and 0.2 mm.; muscles of cirrus sac are 10 to 15μ in thickness. Male reproductive system opens into utero-vaginal canal 0.04 to 0.07 mm. from the outside. Vagina median, convoluted, forming cresent shaped receptaculum seminis 70μ in length before passing over ovarian commissure. Ovarian wings 0.3 to 0.4 mm. long and 0.09 to 0.15 mm. wide. The last third of uterus not surrounded by typical uterine glands; this portion lies anterior to ovarian commissure. Vitellaria with maximum diameter of 0.08 to 0.13 mm. Eight pairs of excretory canals terminate in excretory vesicle 45 to 60μ long by 20 to 25μ wide. Eggs oviform and measure 48 to 54μ in length by 31 to 37μ in width.

Host: *Erimyzon sucetta,* Eno River, North Carolina. In intestine.

Biacetabulum giganteum n. sp. [Figs. 10-13]

Specific diagnosis: With characters of genus. Adults 7 to 16 mm. in length and 0.8 to 1.08 mm. in breadth. Scolex resembling well developed "II" type, bearing four loculi and two well defined acetabular-like suckers. Cuticula 7 to 9μ thick; subcuticula and cortical parenchyma fused measuring 38 to 70μ in thickness; medullary parenchyma occupies three fourths to seven eighths of body width. Inner longitudinal muscles reduced to eight fasiculi in base of scolex; outer longitudinal and cuticular muscles present and prominent. Testes irregularly oval, measuring 0.12 to 0.2 mm. in greatest diameter and number 165 to 215. Vas deferens may fill entire medullary parenchyma; seminal vesicle 0.13 to 0.2 mm. long; ductus ejaculatorius straight and about 0.15 mm. in length. Cirrus sac ovoid, 0.22 to 0.27 mm. in diameter; muscles are thick, measuring 33 to 47μ in thickness. Cirrus sac opens into a utero-vaginal canal 30 to 70μ from the outside. Vagina ventral, straight,

thick walled and forms a receptaculum seminis 0.06 to 0.142 mm. in length. Ovarian wings are 0.37 to 0.54 mm. long and 0.16 to 0.23 mm. wide. Vitellaria surround the testes and measure 0.12 to 0.23 mm. in maximum diameter. Excretory system has 8 pairs of canals; descending canals empty into excretory vesicle 40 to 60μ long. There were no eggs present, although the form appears to be sexually mature.

Host: *Ictiobus bubalus* and Ictiobus sp., Tallahatchie River, Mississippi. In intestine.

References Cited

Baylis, H. A. 1928.—Some parasitic worms, mainly from fishes, from Lake Tanganyika. Ann. Mag. Nat. Hist., 1: 552-562; 6 figs.

Bovien, P. 1926.—Caryophyllaeidae from Java. Vidensk. Medd. Dansk. naturh. Foren., 82: 167-181; 20 figs.

Fuhrmann, O. and Baer, J. G. 1925.—Zoological results of the third Tanganyika expedition by Dr. W. A. Cunnington, 1904-1905. Reports on the Cestoda. Proc. Zool. Soc. London, 1925: 79-100; 14 figs.

Hunter, G. W., III. 1927.—Notes on the Caryophyllaeidae of North America. Jour. Parasit., 14: 16-26; 2 pl.

Hunter, G. W., III. (MS).—Studies on the Caryophyllaeidae of North America. Ill. Biol. Monogr. (in press).

Johnston, T. H. 1924.—An Australian Caryophyllaeid cestode. Linn Soc. New South Wales, 49: 339-347; 17 figs.

Motomura, I. 1927.—On *Caryophyllaeus gotoi,* n. sp., a new monozoic cestode from Korea. Sci. Rep. Tohoku Imp. Univ. (4 ser.), 3: 51-53; 1 fig.

Nybelin, O. 1922.—Anatomische Systematische Studien über Pseudophyllideen. Göteborgs Kgl. Vetensk. Vitterh. Samh. Handl., 26: 1-288; 117 figs.

Will, H. 1893.—Anatomie von *Caryophyllaeus mutabilis* Rud. Ein Beitrag zur Kenntnis der Cestoden. Zeit. wiss. Zool., 56: 1-41; 2 pl.

Wisniewski, L. W. 1928.—*Archigetes cryptobothrius,* n. sp. nebst Angaben über die Entwicklung im Genus Archigetes R. Leuck. Zool. Anz., 77: 113-124; 3 figs.

Woodland, W. N. F. 1923.—On some remarkable new forms of Caryophyllaeidae from the Anglo-Egyptian Sudan, and a revision of the families of Cestodaria. Quart. Jour. Micr. Sci., 67: 435-472; 2 pl.

1924.—On a new species of the Cestodarian genus Caryophyllaeus from an Egyptian Siluroid. Proc. Zool. Soc. London, 1924: 529-532; 4 figs.

1926.—On the genera and possible affinities of the Caryophyllidae: a reply to Drs. O. Fuhrmann and J. G. Baer. Proc. Zool. Soc. London, 1926: 49-69; 8 figs.

Explanation of Plate XIII

a, acetabula-like sucker
cs, cirrus sac
i, inner longitudinal muscles
lc, longitudinal cuticular muscles
olm, outer longitudinal muscles
ot, ootype
s, seminal vesicle
ul, primary limb of uterus
u2, secondary limb of uterus (ascending)
u3, tertiary limb of uterus (descending)
v, vagina
vd, vas deferens
vtd, vitelline duct

Scale in figures 2, 8, 11, 15 and 16, 0.2 mm.; in others, 0.5 mm.

Fig. 1.—*Glaridacris confusus,* toto, scolex. 2. Cross section, through testes. 3. Toto, reproductive systems. Fig. 4. *Pseudolytocestus differtus,* toto, scolex. 5. Cross section, through testes. 6. Toto, reproductive systems.

Fig. 7.—*Spartoides wardi,* toto, scolex. 8. Cross section, through testes. 9. Toto, reproductive systems.

Fig. 10.—*Biacetabulum giganteum,* toto, scolex. 11. Cross section, through scolex. 12. Cross section, through testes. 13. Toto, reproductive system. 14. *Biacetabulum meridianum,* toto, scolex. 15. Cross section, through scolex. 16. Cross section, through testes. 17. Toto, reproductive systems.

PLATE XIII

PHILOMETRA NODULOSA NOV. SPEC.

WITH NOTES ON THE LIFE HISTORY *

LYELL J. THOMAS

During the summer of 1927 at the University of Michigan Biological Station, Douglas Lake, Michigan, while the class in helminthology was examining fish hosts for parasites, a student called the attention of the writer to a peculiar raised and inflamed node on the upper lip of a common sucker, *Catostomus commersonii*. Closer examination showed the outlines of a worm coiled just under the dermis. The nematode was removed and placed in normal salt solution. Viewed under the microscope its uterus was seen to be teeming with active larvae.

The following description of the adult worm is based on a single specimen now No. *29-1* in the ward collection at Urbana. A description of the larvae and infection experiments will be taken up later.

Philometra nodulosa nov. spec. in the living state was glistening white in color and measured 28 mm. long and 0.456 mm. in diameter at the widest part through the mid region. From here the body tapers to form a rounded tip at the anterior end (Fig. 1) and a truncated posterior end (Fig. 2). Highly refringent transverse bosses cover the cuticula. They are irregularly spaced end to end over the surface of the body so as to form a series of incomplete rings which vary in proximity to adjacent rings from 0.004 mm. to 0.124 mm. In general the appearance is similar to that described for *Filaria cingula* Kecker (1916); however, the individual bosses appear more nearly like those shown in *Katanga katangensis* Gedoelst (1916). The bosses project as high as 6μ from the surface and have a length of as much as 41.5μ. In addition to these elevations of the cuticula, minute transverse striations are present but in a glycerine mount are almost obscured by the prominent muscle layer beneath. Three distinct layers of the cuticula were distinguished by means of a modified Mallory triple stain. The outer layer is less than 1μ thick and the combined thickness of the other two is 2μ.

The mouth is terminal and is surrounded by six minute lips (Fig. 3) situated within a shallow depression. The dorsal and ventral lips are somewhat retrorse and slightly larger than the two lateral lips on either side. The depression may be due to a contracted condition but without other specimens for comparison this may be an erroneous view.

The diameter of the body 0.083 mm. from the anterior end is 0.166 mm. but just posterior to a rupture in the body wall a distance of 0.747 mm. from the mouth the diameter increases to 0.216 mm.

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* Contribution from the University of Michigan Biological Station and the Zoology Laboratory of the University of Illinois No. 338.

The esophagus is tripartite with a very muscular bulbous anterior part (Fig. 3) extending down for a distance of 10μ. This expansion measures 49μ in diameter whereas just beyond it the esophagus measures 40μ in diameter and is only weakly muscular. A similar expansion of the esophagus is figured by Ward (1918: 524) in *Ichthyonema cylindraceum* in *Perca flavescens*. Yorke and Maplestone (1926) accept the Genus Philometra Costa, 1845 and consider Ichthyonema Diesing, 1861 as a synonym. Anterior and dorsal to the expansion is a small gland-like body.

Just posterior to the rupture in the body wall the esophagus unites with a capacious though degenerating intestine which is 0.15 mm. in diameter in this region. The anterior part of the intestine is free in the body cavity but gradually becomes smaller in diameter and lies closely applied to the body wall. Sloughed off cell fragments are scattered throughout its lumen. There is no trace of an anus.

The rather short anterior ovary measures 33.2μ wide where it protrudes through the ruptured body wall. The posterior ovary is apparently vestigial. The uterus was packed throughout its length with active larvae and a scattering of ova in various stages of development (Figs. 5-8) together with cast egg envelopes and embryonic debris. The eggs have thin hyaline shells and in the advanced stages of development are ellipsoidal measuring 12 by 14μ.

A few transverse sections 4μ thick were made through the worm slightly posterior to the rupture (Fig. 4). The lateral lines are broad and conspicuous measuring about 14μ wide through the mid body region. The muscle fibers, about fifty-four in number on either side of the dorsal and ventral nerves extend into the body cavity so as to form a muscular ridge on either side of these two nerves. Traces of muscle extending almost to the median line of the lateral fields are brought out by the aid of a modified Mallory triple stain.

DESCRIPTION OF THE LARVAE

The larvae (Fig. 9) just escaped from the uterus are unsheathed and measure about 316μ long and 35μ wide in the mid region. All larvae in the uterus were uniform in size, a condition similar to that described by Mackin (1927) for *Dracunculus globicephalus* in *Chelydra serpentina*. The larvae themselves however more nearly resemble those of *Philometra globiceps* represented by Zur Strassen (1907). At a distance of 2μ from the anterior end they are 6μ in diameter. The body tapers poisteriorly to end in a filiform tail which is studded near the tip with minute papillae. Exceedingly fine transverse striations cover the entire cuticula in addition to a scattering of minute papillae.

The anus is situated on the ventral surface a distance of 48μ from the posterior end. Here also on the ventral surface at the anal opening a

curious protuberance is present in all larvae examined, in those taken from the uterus of the adult worm after it had been killed in hot glycerine and alcohol as well as in those that had escaped from the living worm in well water. The writer is inclined to believe it is a rupture or prolapse with adhering exuviae but because of its occurrence in both the killed and living specimens has not overlooked the possibility of its being a mammelon similar to that described by Bastinian (1863) for the larvae of *Dracunculus medinensis*.

Only two lips were visible, one dorsal and one ventral to the mouth. The anterior end recedes toward the tip by two levels of sharply decreasing diameter, giving it a step effect in profile, similar to that of forms with a prepuce. A thin esophagus in which a lumen is visible occupies the anterior third of the body. Posteriorly, the esophagus joins the expanded intestine which is marked by the presence of highly refringed granules or rhabditin (Cobb, 1914), common to all nematode larvae. Large cells are seen through the intestine wall or above or below throughout its course. These cells may be the developing gonads. The lumen of the lateral line canal opens to the outside ventrally and a little posterior to the nerve ring. Two adjacent dark brown bodies were noted in the region of the intestine midway of the body. Cement glands in the caudal region open to the outside 40μ from the distal end of the tail.

INFECTION EXPERIMENTS WITH LARVAE

Exp. 1.—To discover the intermediate host. On July 9, 1927 at 5 p. m. the adult worm which had been in normal salt solution since 9 a. m. was placed in a stender dish containing well water. Within a few seconds the worm burst, 0.747 mm. from the anterior end and a portion of the ovary and of the esophagus protruded as in Fig. 1 while at the same time thousands of living larvae teemed out of the ruptured wall in a milky cloud. Ten 2 inch stender dishes were filled with about one half inch of well water and then a plankton tow from the lake was added. A few drops of water containing numerous larvae were placed in each of the above dishes. The larvae actively wiggled about or vigorously coiled and uncoiled like a spring. Some seemed to adhere to the bottom of the stender dish by means of their tails and to go through the coiling movements in the one location. Later, examination of the larvae under an oil immersion lens confirmed the presence of cement glands. Cyclops and Daphnia were examined until late that evening to determine if any were injesting worms but without results. The next morning, July 10 at 8 a. m. most of the Daphnia were found to be dead in the dishes but of both living and dead of those examined none were found infected. Out of ten Cyclops examined from one dish four *Cyclops brevispinosus* contained active larvae in the body cavity. One had four active larvae, the others only a single

larvae in each. Ten Cyclops from a second dish showed six infested with larvae. Ten Cyclops from the third dish were examined and two *C. brevispinosus* were found with numerous worms in the body cavity. The fourth dish contained seven infected Cyclops out of ten examined. Both Cyclops and worms were active. All ten Cyclops examined from the fifth dish contained one or more worms in the body cavity. No infected Cyclops were found in a sixth dish. Four infected Cyclops were found in the seventh dish. The lot in the eighth dish was not examined until 4 p. m., July 14; six out of ten Cyclops examined were infected and these were *C. brevispinosus*. Four Daphnia from this same lot did not contain worms. All larvae in the dish were dead. On July 14 a ninth dish was examined. All larvae in the dish were dead and no Cyclops or Daphnia were infested. Three *C. brevispinosus* were found infected in the tenth dish on July 10 and when examined again July 18 all three were alive. One containing seven worms in the body cavity was mounted in glycerine (Fig. 10). When examined again July 24 all Cyclops in the dish were dead. All larvae in the original stender dish when examined at 8 a. m. July 12 were dead. Another plankton tow from the lake was added to this same dish and out of one hundred Cyclops examined from this lot between July 12 and 18 not one infected crustacean was found.

From the above experiment it is evident that an infection of over 50 per cent among the Cyclops was due to rather close contact with numbers of larvae in a limited area. In nature the chances of such heavy infection are slight as is shown by the absence of worms in the hundred Cyclops not exposed. The larvae live only from one to three days unless eaten by a suitable copepod. In this respect they are similar to those larvae from an undescribed guinea-worm infecting the cobra, *Naia tripudiens,* as described by Turkhud (1920). This is also true of the length of life of *Dracunculus medinensis* larvae as shown by the experiments of Leiper (1907). From the behavior of the adult worm in well water it is suggested that under natural conditions a rupture of the node on the lip of the sucker would free the larvae as was done experimentally.

No great increase in size was noted in the larvae infecting the Cyclops although all lots were examined until all the crustacea had died. In the first dish Cyclops and the worms within them were alive on July 16 and active and when examined again July 24 were all dead. In the second dish on July 16 infected Cyclops and worms within them were active. All were dead in this same lot when examined July 26 although they were still alive though sluggish on July 24. On July 19 all Cyclops in the third dish were dead. The fourth dish contained Cyclops and worms still active on July 16 but all were dead on July 24. On this same date Cyclops in the original stender dish were alive and

active. From the above data no definite conclusions may be drawn for it is possible that the unnatural conditions of existence in a small stender dish contributed to the early mortality of the crustacea.

Exp. 2.—To determine the final host, young suckers 27 mm. long were obtained. One was placed in the fifth dish with the heaviest infested lot July 16. On July 18 all Cyclops and other crustacea had disappeared from the dish and the young sucker frightened by the handling of the dish dashed into the side of the dish and soon died. A careful dissection of all parts of the body showed only cestodaria and trematodes in the intestine, one metacercaria in the eye and several encysted along the backbone and tail but no nematodes. Another young sucker placed in dish 7 died July 20 and was also negative for nematodes, although the intestine was infested with larval trematodes and cestodaria. It is possible that sufficient time had not elapsed for the larvae to complete their development in the Cyclops or that the Cyclops were not the right intermediate host. The Cyclops will eat almost any minute moving object; the writer has observed them grabbing up protozoa, coracidia, and in one instance, one of these larval Philometra that coiled and uncoiled in the intestine of the Cyclops until it suddenly broke through into the body cavity. A heavy infestation would undoubtedly damage the intestine of the Cyclops beyond repair and allow bacteria and protozoa to enter the body cavity as has been observed with heavy invasions of coracidia. As the Cyclopidae are cannibalistic the disappearance of crustacea in the dishes may be partly due to this habit. Because of the limited supply of infective larvae it was impossible to follow the life history further.

Although a careful examination was made of all *Catostomus commersonii* used in the laboratory during the summer of 1928 no more *Philometra nodulosa* were found.

Literature Cited

Bastian, H. C. 1863.—On the Structure and Nature of the Dracunculus or Guinea-worm. Trans. Linn. Soc. London, 24: 101-134, pl. 21-22.

Cobb, N. A. 1914.—Rhabditin. Jour. Parasit., 1: 40-41.

Gedoelst, L. 1916.—Notes sur la faune parasitaire du Congo Belge. Rev. Zool. Africaine, 5: 1-90.

Kecker, F. H. 1916. *Filaria cingula* parasitic in the skin of *Cryptobranchus alleghensis*. Jour. Parasit., 2: 74-79.

Leiper, R. T. 1907.—The Etiology and Prophylaxis of Dracontiasis. Brit. Med. Jour., 1: 129-132.

Mackin, J. G. 1927.—*Dracunculus globocephalus* n. sp., from *Chelydra serpentina*. Jour. Parasit., 14: 91-94.

Zur Strassen, O. K. L. 1907.—*Filaria medinensis* und Ichthyonema. Verhandl. deutsch. Zool. Gesellsch., 17: 110-129.

Turkhud, D. A. 1920.—Dracontiasis in Animals. Ind. Jour. Med. Res., 7: 727-734.

Ward, H. B. 1918.—Parasitic roundworms. In Fresh-water Biology. 1111 pp., 1,547 figs. New York.

Yorke, W. and Maplestone, P. A. 1926.—The Nematode Parasites of Vertebrates. 536 pp., 306 figs. Philadelphia.

Explanation of Plate XIV

Camera lucida drawings; scale, 0.05 mm., except in figure 10 where it equals 0.2 mm.

Fig. 1.—Anterior end of female, lateral view, showing bosses on cuticula, lateral line, muscle layers, and portions of ovary and esophagus protruding through ruptured ventral body wall.

Fig. 2.—Posterior end of female, ventral view, showing truncated end with no trace of anus.

Fig. 3.—Tilted ventral view showing lips and portion of esophagus with bulbous muscular part and gland dorsal.

Fig. 4.—Transverse section 4μ thick through female posterior to rupture in body wall, showing ovary, disintegrating intestine, uterus containing section through egg, lateral fields, muscles, and dorsal and ventral nerves.

Figs. 5, 6, 7, and 8.—Eggs taken from uterus in mid-body region in different stages of development.

Fig. 9.—Glycerine mount of larvae taken from uterus showing lips, striations and papillae of cuticula, nerve ring, esophagus, duct of lateral line canal, intestine, protuberance, caudal glands in anal region.

Fig. 10.—Infested *Cyclops brevispinosus* with seven *Philometra nodulosa* larvae in body cavity.

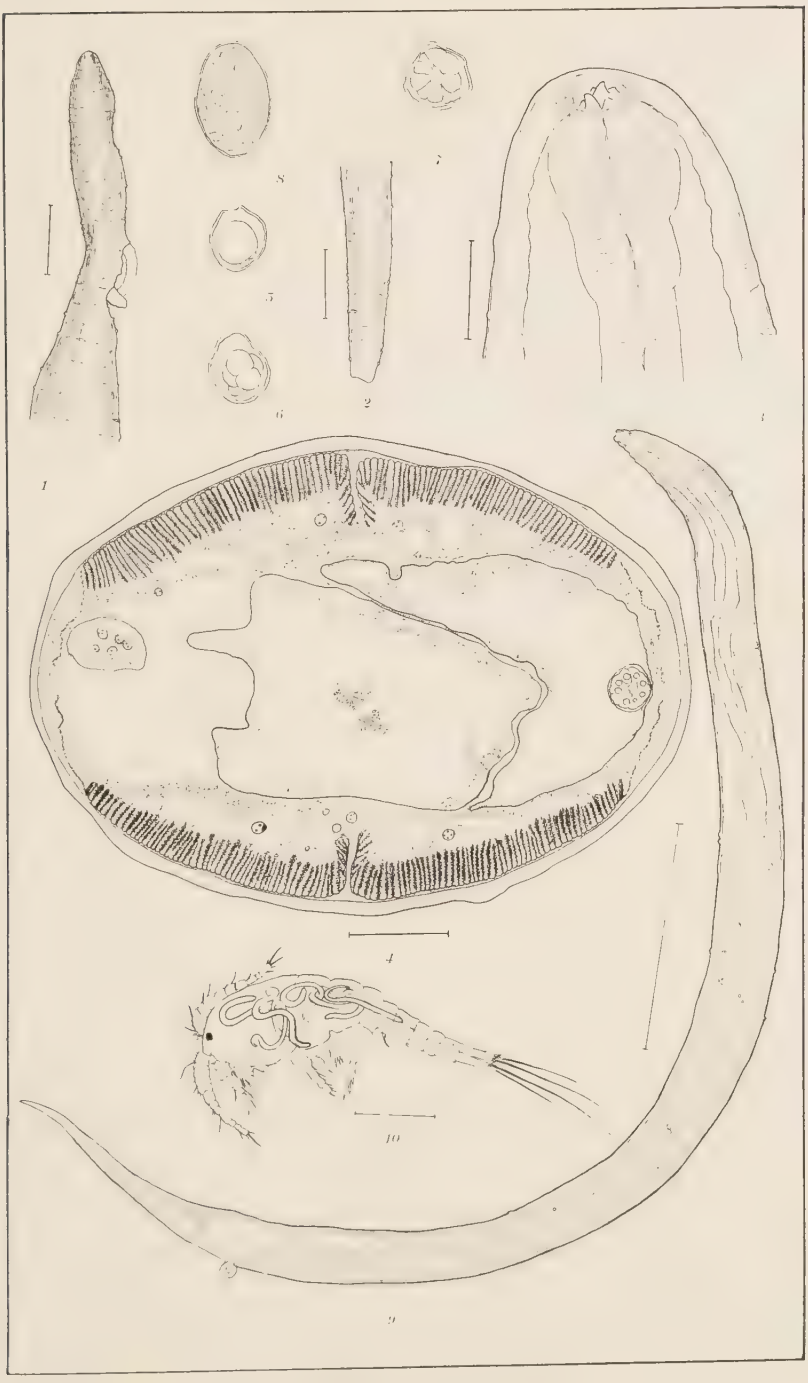


PLATE XIV

NOTES ON CERCARIAE FROM MISSOURI*

Oliver R. McCoy

In a previous paper (McCoy, 1928) the results have been published of life history studies on trematodes from the vicinity of St. Louis, Missouri. During the course of this investigation, observations were made upon a number of cercariae whose life histories were not solved. It is the purpose of this paper to describe four of these cercariae as new species, and to fix the excretory system of another described species, a xiphidiocercaria, *C. hemilophura* Cort, 1914. One of the new species, *Cercaria brevifurca,* is of interest because it is the first lophocercous fork-tailed cercaria to be reported from the fresh waters of North America.

As an indication of the abundance and variety of the larval trematode fauna in the vicinity of St. Louis, it may be noted that in a two-year survey of approximately 15,000 snails of the genera, Planorbis, Physa and Lymnaea, from various localities, a total of 21 species of cercariae were found, at least 12 of which have never been described. These species include 9 xiphidiocercariae, 3 echinostomes, 2 amphistomes, 1 monostome, 4 furcocercous, 1 gymnocephalous distome cercaria, and 1 cercariaeum. The percentage of infected snails as a rule was very low, seldom exceeding 10 per cent. A complete record of the seasonal infestation of two of the species in *Planorbis trivolvis* has been published (McCoy, 1928*a*), and it is not considered significant to include in this paper isolated data on the incidence of infestation of the species described.

Practically all observations were made upon living specimens which had emerged from the snail host. Snails collected in the field were brought into the laboratory and isolated the same day in a small amount of water in glass vials. If mature cercariae were present in the snail, they usually emerged within 24 hours and with the aid of a hand lens could easily be seen swimming about in the water. After a supply of cercariae had been obtained for study, the snails were killed and dissected to obtain the parthenitae. If no cercariae emerged from the snails within 48 hours, the snails were dissected and the immature infestations detected. All four of the species described were found a number of times so that there was ample material for study. The measurements cited are average measurements of 6 to 12 specimens of each species killed with gentle heat and mounted under a cover glass

* From the Zoological Laboratory of Washington University, St. Louis, Mo. The writer is indebted to Dr. H. M. Miller, Jr., for many helpful suggestions and criticisms.

so that practically no pressure was exerted upon the animal. Since cercariae show great variation in shape and degree of extension, size measurements are not stressed as specific characters.

Cercaria missouriensis, n. sp. [Fig. 1]

Cercaria missouriensis was found on numerous occasions in *Planorbis trivolvis* in all months of the year from March to September. It is an amphistome cercaria belonging to the Diplocotylea Group established by Sewell (1922:80). Sewell named six species as belonging to this group but omitted the mention of *C. convoluta* Faust, 1919, a form which closely resembles the present species, and should undoubtedly be included in the group.

C. missouriensis is a large form, measuring 0.575 by 0.325 mm. The body, however, is very extensile and frequently is contracted into a round ball. The tail is normally about twice as long as the body, being 1.12 by 0.13 mm. The oral sucker measures 0.1 by 0.09 mm., and a pair of thin-walled retractile pharyngeal pouches are located at its posterior border. The prepharynx is short, and the ceca branch out immediately from the pharynx and extend nearly to the acetabulum; they are wide, thick-walled and empty, and often difficult to distinguish in the living specimen. The acetabulum occupies the whole posterior end of the body and is very large, measuring 0.16 by 0.25 mm.

A pair of well-defined pigmented eye-spots, 0.054 by 0.03 mm., are present dorsal and lateral to the pharynx, and pigment is thickly distributed in small patches over the dorsal surface of the body anterior to the acetabulum. The body contains numerous cystogenous glands in which the material is arranged in rod-like packets. The cercariae have often been observed to drop their tails and encyst under a cover-glass but have never been observed to do so in open vials. Immediately anterior to the excretory vesicle are two masses of cells which probably represent the beginning of the reproductive system.

A small excretory vesicle is situated just anterior to the acetabulum and from it, a long canal extends into the tail forking near the end. The main excretory tubules are only slightly coiled, and in the region near the anterior end of the body, are distended by a few large globular concretions. The number of these large concretions is remarkably constant, six to eight, and since no exceptions were noticed, their presence may even be considered a specific character. The main tubules loop back near the anterior sucker and shortly receive anterior collecting tubules; they then continue posteriorly to the acetabulum breaking up into numerous smaller tubules and capillaries.

The cercariae develop in large rediae (Fig. 2) which measure 1.4 by 0.35 mm. There is a pharynx 0.055 mm. in diameter, and a thin-walled gut which extends approximately one-fourth the length of the

body. Two pairs of locomotor appendages are present in the posterior half of the body, the second pair appearing very indistinctly. Apparently the cercariae do not undergo complete development in the rediae, for many immature forms were present free in the tissues of the snail. This observation has been made in other species of this group.

C. missouriensis is similar to both *C. inhabilis* Cort, 1914, and *C. diastropha* Cort, 1914, in all general characters but most closely resembles *C. convoluta* Faust, 1919. It differs from the latter chiefly in the greater size of its acetabulum which is nearly half again as large as that of *C. convoluta,* and also there are differences in the excretory system; the main tubules of *C. convoluta* are very much coiled and contain no concretions, the excretory tubule in the tail forks farther anterior than in *C. missouriensis,* and the branches do not empty as close to the end of the tail.

Specific diagnosis. *Cercaria missouriensis.*—Amphistome cercaria from *Planorbis trivolvis.* Very extensile body, 0.575 by 0.325 mm.; tail about twice as long as body. Acetabulum large, 0.16 by 0.25 mm. Pair of retractile pharyngeal pouches present; prepharynx short; large, empty, thick-walled ceca branching out immediately from pharynx, and extending nearly to acetabulum. Well-defined, pigmented eyespots present dorsal and lateral to pharynx. Pigment distributed over dorsal surface of body anterior to acetabulum. Cystogenous glands with rod-like contents. Main excretory tubules near anterior end of body distended by a few large concretions, remarkably constant in number, 6 to 8. Development in rediae with short gut extending about one-fourth length of body; two pairs of locomotor appendages present, posterior pair indistinct.

Cercaria rebstocki, n. sp. [Fig. 3] *

On numerous occasions an echinostome cercaria was found in *Planorbis trivolvis* which is very similar to, if not identical with *C. complexa* Faust, 1919. Since, however, there is an incompatible difference in the excretory systems of the two forms and several other smaller differences, the cercaria is here described as a new species, *C. rebstocki.* It belongs to the Coronata Group of echinostome cercariae the characters of which are described by Sewell (1922: 116). In his original text Sewell defines the group as developing in rediae which possess a long wide gut reaching the posterior end of the body, but in a footnote (p. 117) he modifies this definition to permit the inclusion of the cercaria of *Echinostoma revolutum* which develops in a redia with a short gut. *C. rebstocki* is another member of this group whose redia possesses a short rhabdocoele gut.

C. rebstocki resembles other described echinostome cercariae in all general characters. The body measures 0.37 by 0.175 mm. and possesses a collar of 37 spines arranged in a double row interrupted ventrally. The spines are difficult to count accurately, but in most

* This species is named as a compliment to the late Mr. Charles Rebstock, donor of Rebstock Hall of Biology, Washington University.

specimens 19 were distinguished across the dorsal surface and 9 on each ventral shoulder. The tail is slightly longer than the body, 0.48 by 0.041 mm. The oral sucker measures 0.057 mm. in diameter and the ventral sucker is slightly larger, 0.061 mm. in diameter. There is a short prepharynx and a pharynx, 0.029 by 0.023 mm.; the esophagus forks anterior to the ventral sucker and the narrow ceca extend nearly to the posterior end of the body. The entire body posterior to the collar is filled with cystogenous glands with rod-like contents. There are four pairs of gland ducts opening on the dorsal lip of the mouth. The outermost duct on each side opens far out from the others, which are located close together near the midline. The glands from which these ducts lead were not definitely located but are probably situated along the esophagus.

There is a small excretory vesicle in the posterior part of the body from which a canal extends into the tail, forking at about one-sixth the length of the tail. The main excretory tubules are filled with large concretions from the pharynx to the ventral sucker; they loop back on themselves anterior to the pharynx, and at the posterior margin of the ventral sucker receive anterior and posterior collecting tubules. Further details of the excretory system were not determined with certainty, but there are approximately 15 flame cells on each side of the body. The three most anterior flame cells, as is typical in other echinostome cercariae, are arranged in a triangle in the region anterior to the pharynx and drain into the anterior collecting tubule. The cercariae develop in small rediae (Fig. 4), 0.85 by 0.25 mm., which possess a pharynx, 0.04 mm. in diameter, and a very short gut which does not extend far posterior to the collar. There is a pair of locomotor appendages on the posterior third of the body, and a birth pore posterior to the collar.

C. rebstocki is distinguished from *C. complexa* Faust, 1919, as a new species because it has 37 collar spines instead of 38, 4 pairs of gland ducts opening on the dorsal lip of the mouth instead of 3 pairs, and because the secondary portion of the excretory tubule receives branches in the region of the ventral sucker, whereas in *C. complexa* the secondary portion of the tubule continues to the posterior end of the body unbranched. Cysts of *C. rebstocki* have been found naturally in the mantle cavity of *Planorbis trivolvis* and *Physa integra,* but they occur more often and in greater numbers in the latter. The cercariae have also been found experimentally to encyst in both species of snails. The cysts are spherical and measure 0.136 mm. in outside diameter. The cyst wall is transparent and the general features of the encysted cercaria may be easily distinguished. Cysts from laboratory infested snails were fed to young chicks and ducklings but no adults were recovered.

Specific Diagnosis. *C. rebstocki.*—Echinostome cercaria from *Planorbis trivolvis* with collar of 37 spines arranged in a double row. Body 0.37 by 0.175 mm.; tail slightly longer than body. Prepharynx short, esophagus forking anterior to ventral sucker, and ceca extending to end of body. Body filled with cystogenous glands with rod-like contents. Four pairs of gland ducts opening on dorsal lip of mouth. Main excretory tubules filled with concretions from pharynx to ventral sucker, looping back from pharynx and receiving just posterior to ventral sucker, anterior and posterior collecting tubules. Approximately 15 flame cells on each side of body. Development in rediae with very short gut not extending far past collar.

Cercaria longistyla, n. sp. [Fig. 5]

Cercaria longistyla is a small cercaria with a very long stylet in proportion to the size of the body, and belongs to the ornate group of xiphidiocercariae. This group was created by Lühe and is characterized by the presence of a fin-fold on the tail. Only a few cercariae of this type have been described and the group is probably an unnatural one. *C. longistyla* belongs in a sub-group, the Prima Group defined by Sewell (1922: 219) and is the first larva of this type to be described from North America. The other members of this sub-group are *C. prima* Ssinitzin and *Cercariae indicae* XXIV and XXVIII Sewell. *C. hemilophura* Cort, 1914, and *C. trifurcata* Faust, 1919, the two ornate xiphidiocercariae hitherto reported from North America, on account of their more highly developed excretory systems, cannot be included in the Prima Group.

The body of *C. longistyla* is about 0.32 by 0.15 mm. and is entirely covered with small spines. The tail is narrow and stumpy, and is a little less than half as long as the body. On its distal end there is a narrow dorso-ventral fin-fold which ventrally is wider and extends further anterior than on the dorsal side. The stylet is very long and narrow, measuring 32 by 4μ and has a thickening about one-fourth its length from the point (Fig. 6). The oral sucker is 0.065 mm. in diameter and is much larger than the ventral sucker located in the posterior third of the body, which measures only 0.04 mm. in diameter. There is a short prepharynx, and a pharynx, 0.019 mm. in diameter, from which a narrow esophagus extends to about the middle of the body where it forks into two ceca which extend to the posterior end. The ceca are narrow, inconspicuous, and difficult to trace.

Five granular stylet glands are present on each side of the body anterior and lateral to the ventral sucker. The two anterior glands of each group are coarsely granular and do not stain with neutral red; the three posterior glands, however, are more finely granular and take a dark red stain. Ducts from these glands pass forward to empty at the side of the stylet. The body contains numerous other small glands probably cystogenous in charatcer. The excretory vesicle is thick-walled and "Y" shaped, the arms of the "Y" spreading laterally and not extending to the ventral sucker. The ends of the arms receive

the main collecting tubules which are greatly coiled and at the posterior margin of the ventral sucker receive anterior and posterior collecting tubules. Sewell (1922) found the excretory systems of *C. indicae* XXIV and XXVIII to be of the "2 × 6 × 1" type; the accessory collecting tubules in *C. longistyla* conform to this type, but since they were not each definitely traced to a single flame cell, the exact pattern cannot be stated with certainty.

C. longistyla develops in small typical oval sporocysts, which have been found in *Physa integra* and *Physa anatina* in the months of June and July only. The cercariae have been observed to encyst in dragon fly larvae and small water insects experimentally exposed to infection, but apparently they will not encyst in tadpoles. The cysts are thin-walled and measure about 0.145 by 0.13 mm. The contained metacercaria differs from the cercaria in that the stylet and tail have been discarded, the stylet glands have disintegrated, and the excretory vesicle has become filled with numerous small refractile concretions.

Specific Diagnosis. *C. longistyla*.—Ornate xiphidiocercaria from *Physa integra* and *Physa anatina* with body 0.32 by 0.15 mm. and entirely covered with small spines. Tail stumpy, less than half as long as body, with narrow fin-fold on distal end. Stylet long and narrow, 32 by 4μ, with thickening about one fourth its length from point. Small pharynx; esophagus forking near middle of body, and ceca extending to posterior end. Five stylet glands on each side of body anterior and lateral to ventral surface. Two anterior glands of each group coarsely granular, not staining with neutral red; three posterior glands finely granular, taking stain. Excretory vesicle thick-walled and "Y" shaped, arms of "Y" spreading laterally and not extending to ventral sucker. Development in typical oval sporocysts.

Cercaria brevifurca, n. sp. [Fig. 7]

Cercaria brevifurca is a furcocercous cercaria of the lophocercous type, a group characterized by the presence of a median dorsal fin-fold on the body. The name of the present species was suggested by the proportionately short length of the divisions of the tail. Sewell (1922) described four larvae of the lophocercous type and defined the characteristics of the group in part as follows: no trace of pharynx or intestine; formula of excretory system apparently "2 × 3 × 1"; development in small oval or rounded sporocysts. Cercariae belonging to the genus Sanguinicola which have recently been studied by Scheuring (1923) and Ejsmont (1926), would fit into the grouping of Sewell except that they possess a rhabdocoele gut. Also, Ejsmont describes two excretory canals extending from the excretory vesicle into the tail stem, whereas in all four of Sewell's forms there was a single canal in the tail stem which divided at the point where the tail forked.

Another cercariae of the lophocercous type which presents still further variations is *C. bombayensis* No. 8 described by Soparkar (1921). This larva also possesses a rhabdocoele gut but differs from the other forms in that it develops in small rediae with a definite pharynx and gut. The present species, *C. brevifurca,* closely resembles *C. bombayensis* No. 8 but there are certain differences which warrant its description as a new species, most notably the pattern of the excretory system.

C. brevifurca is the first lophocercous cercaria to be reported from the fresh waters of North America; Linton (1915), however, has reported an undescribed marine species from the Woods Hole region.

C. brevifurca is approximately the same size as *C. bombayensis* No. 8. The body measures 0.14 by 0.038 mm.; the tail stem is 0.282 by 0.026 mm.; and the furci are 76 by 17μ. The furci are slightly constricted off from the tail stem, and near their end narrow abruptly to a slender point. Both tail stem and furci are finely spined. The body possesses the typical median dorsal fin-fold which extends from the posterior end of the body nearly to the region of the anterior organ, and at its broadest point posteriorly is about one-fourth the width of the body. No fin-folds were observed on the furci, a condition contrary to that found in *C. bombayenis* No. 8 and other lophocercous cercariae. Slightly anterior to the middle of the body are a pair of darkly pigmented eye spots; they are cup-shaped with the open side directed anteriorly and dorsally.

The anterior organ, which is about 44 by 21μ, is definitely separated from the body but does not have the appearance of a typical trematode sucker. Its anterior half is heavily spined and may be protruded and retracted. A very small mouth is located ventrally about the middle of the anterior organ; no pharynx is present. The esophagus, which is very narrow anteriorly, gradually widens and posterior to the eye spots curves dorsally to end in an expanded cecum. There are four pairs of small granular glands located in the mid-region of the body ventral to the cecum. Ducts from these glands pass forward on each side of the body and empty together at the tip of the protrusible snout. No spines such as are described by Soparkar in *C. bombayensis* No. 8 were observed to cap the openings of these ducts. In the posterior part of the body are two large cell masses both of which the writer interprets to represent the anlage of the reproductive system. Soparkar believed a posterior cell mass in *C. bombayensis* No. 8 to represent the rudiment of a ventral sucker, but in *C. brevifurca* there is no apparent justification for this belief.

The excretory system (Fig. 8) consists of a very small excretory vesicle located in the extreme posterior end of the body. From it a single canal extends into the tail, forking into the furci and opening to

the outside at their tips. The main collecting tubules coil anteriorly from the excretory vesicle and at about the middle of the body divide into anterior and posterior collecting tubules. The anterior tubule drains a pair of flame cells, one located anterior to the eye spots, and the other posterior to them. The posterior tubule collects from a single flame located at the level of the excretory vesicle. No flame cells were distinguished in the tail, hence the formula of the excretory system is $2 \times 3 \times 1$. In *C. bombayensis* No. 8 Soparkar found four pairs of flame cells in the body and an additional pair in the tail. The $2 \times 3 \times 1$ pattern of *C. brevifurca* agrees with the only other excretory systems completley described in lophocercous cercariae, namely those of *C. indicae* IX and LV Sewell, 1922.

C. brevifurca has been found in *Planorbis trivolvis* developing in simple rediae (Fig. 9), whitish in color, with no collar nor locomotor appendages, but with a prominent pharynx, 0.04 mm. in diameter, and a gut which reaches nearly to the end of the body. The rediae vary greatly in size, one of the largest measuring 1.3 by 0.2 mm.; others about half this size have been observed to contain nearly fully-developed cercariae. The rediae tend to assume a crescentic shape when teased free from the digestive gland of the snail.

Specific Diagnosis. *C. brevifurca*, n. sp.—Lophocercous fork-tailed cercaria from *Planorbis trivolvis* with typical median dorsal fin-fold on body extending from posterior end nearly to anterior organ. Tail stem twice as long as body, with short furci not bearing fin-folds. Tail stem and furci finely spined. Darkly pigmented eye spots anterior to middle of body. Anterior portion of anterior organ heavily spined and in form of a protrusible snout. Mouth ventral, about middle of anterior organ; no pharynx; esophagus ending in expanded cecum posterior to eye spots. Four pairs of glands ventral to cecum with ducts emptying at the tip of protrusible snout. Small excretory vesicle in posterior portion of body with canal extending into tail, forking, and emptying at tips of furci. Excretory pattern of $2 \times 3 \times 1$ type with no flame cells in tail. Development in simple rediae with no collar nor locomotor appendages, but with gut extending nearly to end of body.

Excretory System of *Cercaria hemilophura*, Cort, 1914 [Fig. 10]

On numerous occasions, a xiphidiocercaria was found in *Physa integra* and *Physa anatina* which was identified as *Cercaria hemilophura* Cort, 1914. This larva is characterized by the possession of a ventral fin-fold along the posterior half of the tail. Cort, in the description of the species, only partly figured the excretory system. In the present study made upon an abundance of favorable living material, this system was worked out in detail and found to be of the general type, $2 \times 6 \times 3$, or $2\ [(3 + 3 + 3) + (3 + 3 + 3)]$. Faust, in his synoptic table of flame cell formulae for trematodes (1924, Table II), gave $2\ [(3 + 3) + (3 + 3 + 3)]$ as the formula for his newly created Hemilophura Group of Xiphidiocercariae which contains *C. hemi-*

lophura and *C. trifurcata* Faust, 1919. The present study makes it necessary to remove *C. hemilophura* from this group.

The excretory system is essentially the same as that of *C. polyadena* Cort, 1914, described by Cort (1919), the chief difference being that in *C. polyadena* there are only two flame cells in the most posterior group, whereas in *C. hemilophura* there are three. The main excretory tubules which enter the bladder at the anterior end are considerably coiled, and at the level of the ventral sucker, receive anterior and posterior collecting tubules. Each anterior tubule passes ventral to the digestive cecum and receives three accessory collecting tubules, each of which in turn receives three capillaries, which were traced to flame cells. The number and arrangement of the flame cell capillaries of the posterior collecting tubules are exactly like those of the anterior ones. Consequently there is a total of 36 flame cells, arranged in twelve groups, six on each side of the body. In *C. hemilophura* it is invariable in each flame cell group that the accessory collecting tubule is composed of two divisions, one of which receives a capillary from a single flame cell on the ventral side of the body, and the other receives two capillaries from a pair of dorsal flame cells. In *C. polyadena* the arrangement is not so regular; the paired flame cells are always on the same side of the body but may be either dorsal or ventral.

The exact location of the flame cells in *C. hemilophura* varied greatly in different individuals, probably depending upon the way in which the animal was compressed. The second flame cell group from the anterior end was the most difficult to locate, and without careful study of abundant material might be entirely overlooked.

SUMMARY

1. Four new species of cercariae are described from snails from the vicinity of St. Louis, Missouri: an amphistome, *Cercaria missouriensis,* an echinostome, *C. rebstocki;* a xiphidiocercaria, *C. longistyla,* and a lophocercous fork-tail cercaria, *C. brevifurca.*

2. *C. brevifurca* is the first lophocercous cercaria reported from the fresh waters of North America.

3. *C. longistyla* is an ornate xiphidiocercaria belonging to the Prima Group of Sewell (1922) hitherto unrepresented in North America.

4. The excretory system of another ornate xiphidiocercaria, *C. hemilophura* Cort, 1914, was determined to be of the $2 \times 6 \times 3$ type, which necessitates its removal from the Hemilophura Group proposed by Faust.

REFERENCES CITED

Cort, W. W. 1914.—Larval trematodes from North American fresh water snails. Jour. Parasit., 1: 65-84.

1915.—Some North American larval trematodes. Ill. Biol. Monog., 1: 447-532, 8 pl.

1919.—The excretory system of a stylet cercaria. Univ. Cal. Pub. Zool., 19: 275-281, 1 tf.

Ejsmont, L. 1926.—Morphologische, systematische und entwickelungsgeschichtliche Untersuchungen an Arten des Genus Sanguinicola Plehn. Bull. Acad. Polonaise Sc. Lettres—Serie B, 1925: 877-966, 4 pl.

Faust, E. C. 1919.—The excretory system in Digenea. I. Notes on the excretory system of an Amphistome, *Cercaria convoluta,* nov. spec. Biol. Bull., 36: 315-321.

1919.—The excretory system in Digenea. II. Observations on the excretory system in Distome cercariae. Biol. Bull., 36: 322-339.

1924.—Notes on larval flukes from China. II. Studies on some larval flukes from the Central and South Coast Provinces of China. Amer. Jour. Hyg., 4: 241-300, 2 pl.

Linton, E. 1915.—Sporocysts in an Annelid. Biol. Bull., 28: 115-118.

McCoy, O. R. 1928.—Life history studies on trematodes from Missouri. Jour. Parasit., 14: 207-228, 1 pl.

1928*a*.—Seasonal fluctuation in the infestation of *Planorbis trivolvis* with larval trematodes. Jour. Parasit., 15: 121-126.

Scheuring, L. 1922.—Der Lebenscyklus von *Sanguinicola inermis* Plehn. Zool. Jahrb., Anat., 44: 265-310, 1 pl.

Sewell, Seymour. 1922.—Cercariae Indicae. Ind. Jour. Med. Res., 10: 1-372, 32 pl.

Soparkar, M. B. 1921.—Notes on some furcocercous cercariae from Bombay. Ind. Jour. Med. Res., 9: 23-32, 4 pl.

Explanation of Plate XV

All figures are diagramatic free-hand drawings of living specimens. Scale 0.1 mm. in all except Figures 6 and 7 where it equals 0.01 mm.

Abbreviations: *ao,* anterior organ; *c,* cecum; *e,* eye spot; *f,* fin-fold; *m,* mouth; *r,* reproductive anlage.

1. *Cercaria missouriensis* (cystogenous glands and pigment omitted). 2. Redia of *C. missouriensis.* 3. *Cercaria rebstocki* (cystogenous glands omitted). 4. Redia of *C. rebstocki.* 5. *Cercaria longistyla.* 6. Stylet of *C. longistyla.* 7. Lateral view of body of *Cercaria brevifurca.* 8. Excretory system of *C. brevifurca.* 9. Redia of *C. brevifurca.* 10. Excretory system of *Cercaria hemilophura*—ventral view.

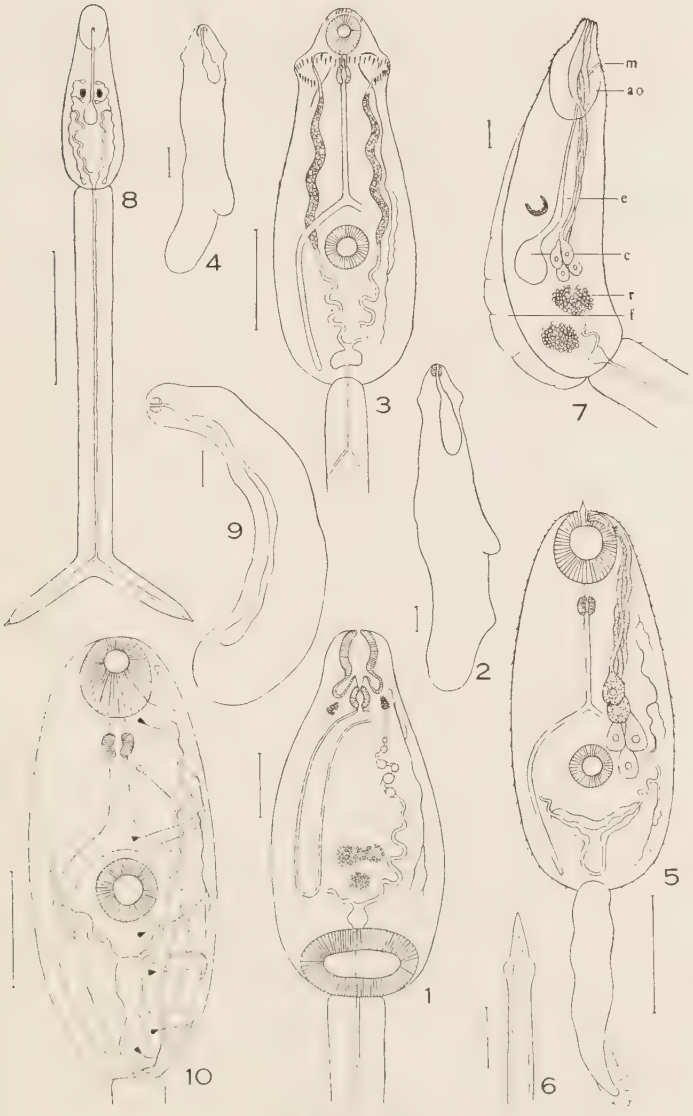


PLATE XV

EXPERIMENTAL DEMONSTRATION OF A STRAIN OF THE DOG HOOKWORM, *ANCYLOSTOMA CANINUM*, ESPECIALLY ADAPTED TO THE CAT.*

J. Allen Scott

So-called physiological species of parasites, distinct by reason of the degree of their infectivity for different hosts, but indistinguishable morphologically are being more frequently mentioned in recent helminthological literature. Experimental demonstration of their exact status has offered certain rather serious difficulties. The case which has commanded most attention is perhaps that of the ascarids of man and of the pig. Enough has been done to indicate that the postulation of physiological strains offers the most plausible explanation of the facts of the epidemiology and distribution of these parasites. Adequately controlled experiments sufficient to satisfy the strictest experimentalist still seem to many to be desirable. Certain difficulties other than the necessity of using man as the experimental animal seem to be involved in this case. This may prove to be true also in the case of attempts to differentiate the trichurids of these same hosts which Schwartz (1926a) has shown to be morphologically identical. Other cases where the same situation has been suspected either have not been tested experimentally or have not yielded readily to this type of treatment.

In the case reported here, however, a situation has fortunately arisen which has admitted of relatively easy experimental analysis. In September, 1925, a cat obtained at Cold Spring Harbor, Long Island, died in this laboratory. Fecal examinations had previously shown the presence of hookworm ova, and at autopsy there were obtained 10 typical specimens of *A. caninum*. The contents of the rectum when cultured yielded larvae which upon being fed to a young dog showed a peculiar lack of infectivity. Larvae from the original cultures were fed to cats and the strain has been kept in cats since that time. The series of experiments described below shows that this strain is as well adapted to cats as a strain obtained from a Baltimore dog is adapted to dogs. But either strain is only slightly infective to the species of host other than the one from which it was obtained. The adults of the two strains are morphologically identical. A series of measurements to show the size relations are now being made.

The results of these experiments serve to explain some of the peculiarities of the distribution of this parasite. It was previously

* Contribution from the department of Heminthology of the School of Hygiene and Public Health of the Johns Hopkins University. The work was carried out with cooperation of the International Health Division of the Rockefeller Foundation.

reported, Scott (1928), that hookworms had never been found in Baltimore cats received in this laboratory although several hundred had been examined, while *A. caninum* was present in 15 or more of about 100 local dogs. Since that time about 100 more cats have been examined but no hookworms have been found, while nearly an equal number of dogs have been found to be infested to about the same extent as previously reported. The literature regarding the presence of this parasite in cats is in a very unsatisfactory state. A few definite reports of its prevalence in cats as compared to dogs in the same locality are available. But for the most part the references are to its occurrence in an occasional animal or a statement that it is found in cats in the region specified. It may be well to mention a few of the more specific of these reports.

Chandler (1925) stated that *A. caninum* was absent from 150 cats in Calcutta although it was present in dogs. He found that it was, however, present in cats in Bengal. Wharton (1917) found this species present in 114 of 118 dogs in the Philippine Islands, and states that it was also common in cats around Manila. Tubangui (1925) recorded this species as occurring in both cats and dogs in Los Baños, Luzon. Schwartz (1924) stated on the other hand that all the hookworms which he examined from cats of the Philippine Islands were *A. braziliense*. In Brazil, Gordon and Young (1922), found *A. caninum* present in 5 out of 9 cats examined in Manaos, and also in all of 50 dogs. Wolfflügel (1911) has recorded one specimen from one cat in Argentina. Adler (1922) found 3 out of 6 cats examined in Freetown harboring this parasite, and also records it as present in dogs. Ortlepp (1926) found 7 specimens in one cat on a South African farm. Schwartz (1926) stated that *A. caninum* has been recorded from China from cats in Peking in 1920 and in 1922, also in Yu Tao Ho in 1920. Its presence is also reported in dogs in China. Maxwell (1921) found it absent from one cat examined in South Fukien, while Faust (1921) does not mention this species as occurring in cats in a report on the parasites of vertebrates in North China. In Australia, Johnston (1916) referred to its having been recorded from one cat in North Queensland and from one in South Queensland. Hall (1928) reported *A. caninum* as present in both cats and dogs in Nicaragua.

In regard to the United States, the literature apparently does not contain any extensive exact references to the occurrence of *A. caninum* in cats, although the fact that it commonly does occur seems to be taken for granted by most writers. The report of its occurrence in 100 of 300 dogs examined in Detroit by Hall (1918) and in 54 of 76 dogs in Washington, D. C. (Hall, 1917) are typical of reports of its occurrence in dogs in this country. Scattered reports of its occurrence in dogs in various parts of the world are much more numerous than the reports of its occurrence in cats.

Although a survey of the available literature yields a small amount of exact information, it does lead to the conclusion that *A. caninum* occurs in cats practically all over the tropical and temperate world. It does not appear to be so common in this host as it is in dogs, and a few references definitely state that it is absent from cats in localities where it is commonly present in dogs. Besides this, it is occasionally reported from various wild Canidae and Felidae. After a presentation of the experimental evidence in regard to the strains here considered, it will be seen that a tentative explanation of the situation may be offered.

EXPERIMENTAL OBSERVATIONS

The experiments deal, as mentioned above, with two strains of morphologically identical parasites, *Ancylostoma caninum* (Erc. 1859). These were obtained from a Baltimore dog and a Long Island cat and will be referred to respectively as the dog strain and the cat strain. The animals for the experiments, after having been kept in the laboratory long enough to allow any young parasites which they harbored to grow to maturity, were all found negative for hookworm ova by Lane's direct centrifugal flotation method. This method will indicate the presence of a single mature female. As far as possible the animals of each litter used were inoculated with both strains of parasites and a control animal kept from the same litter. Except in the one case noted, they were kept in individual cages under conditions which have been adequately demonstrated to prevent accidental infestation. The diet consisted of pork lungs, whole wheat bread, and whole milk. Animals indicated as "pups" and "kittens" were from 2 to 3 months old. "Cats" were mature animals.

The larvae from charcoal cultures were counted and administered orally in gelatin capsules so that no irritation of the mucous membrane occurred until the stomach was reached. This practically eliminated the tendency to vomit when the larvae were given in numbers here used. It is possible that vomiting did occur after observation ceased but previous experience indicates that this is not probable.

The results of the experiments are shown in Table 1. The exact status can best be seen in the averages from kittens and pups. An average of 45 to 50 per cent of the parasites matured in young animals of the same species from which the strains were derived, while of the cat strain in the pups only 0.4 per cent matured, and of the dog strain in the kittens 4.6 per cent matured. Attention should be called to the fact mentioned above that in many cases litters were divided and inoculated at the same time with both strains, larvae from the same culture being used for both cats and dogs. Control animals from most of these litters were kept in identical conditions, but are not tabulated as they were consistently uninfected. The litters to

which the animals belong and the source of the larvae used for inoculation are listed in Table 2.

Mention should be made of certain special cases which have a bearing on the interpretation of the results. Kitten 93 which had less infec-

TABLE 1.—Infectivity to dogs and cats of larvae of *A. caninum* obtained from dog and cat respectively

Experimental Animal	Days after Infection	Number Larvae Given	Number Developed	Per Cent Developed	Experimental Animal	Days after Infection	Number Larvae Given	Number Developed	Per Cent Developed
Cat Strain									
Kitten 93	12	532	92	17	Pup 332	12	371	0	0
Kitten 338	12	403	197	49	Pup 126	13	547	0	0
Kitten 340	15	362	255	70	Pup 127	13	517	2	0.4
Kitten 88	18	496	174	35	Pup 331	21	367	3	0.8
Kitten 90	18	529	192	36	Pup 333	49	355	3	0.9
Kitten 337	19	367	192	52					
Kitten 86	24	518	173	33	Average				0.4
Kitten 345	25	352	235	67					
Cat 302	60	450	76	17					
Cat 106	108	310	55	18					
Average (kittens)				45					
Average (all)				39					
Dog Strain									
Kitten 85	14	376	0	0	Pup 321	9	121	70	58
Kitten 91	18	523	3	0.5	Pup 335	12	384	255	66
Kitten 339	20	393	62	16	Pup 125	12	503	174	35
Kitten 344	21	264	13	5	Pup 124	13	475	100	21
Kitten 92	24	310	9	3	Pup 330	21	447	228	51
Kitten 346	49	265	7	3	Pup 334	38	370	246	66
Cat 305	20	23000	0	0					
Cat 311	35	5240	0	0	Average				50
Cat 312	37	24000	0	0					
Average (kittens)				4.6					
Average (all)				3.0					

TABLE 2.—*Arrangement of experimental animals in litters and cultures used for inoculation*

Litter A.	Dog strain culture 1;	kitten 85
	Cat strain culture 1;	kittens 86, 88
	Control	kitten 89
Litter B.	Dog strain culture, 1;	kittens 91, 92
	Cat strain culture 1;	kittens 90, 93
	Control	kitten 94
Litter C.	Dog strain culture 1;	pups 124, 125
	Cat strain culture 1;	pups 126, 127
Litter D.	Dog strain culture 2;	kitten 346
	Cat strain culture 2;	kitten 345
Litter E.	Dog strain culture 2;	kitten 339
	Cat strain culture 2;	kittens 338, 340
	Control	kitten 342
Litter F.	Dog strain culture 2;	pups 330, 334, 335
	Cat strain culture 2;	pups 331, 332, 333
Others	Dog strain culture 2;	pup 321, kitten 344
	Cat strain culture 2;	kitten 337
Others inoculated individually with various cultures		

tion than others of the same class died when the worms were very small and consequently difficult to recover at autopsy. This may explain the low percentage obtained, although Kitten 338 died at the same period. As will be shown in a later paper the worms in dogs grew so much more rapidly that there was no difficulty in recovering any in this series. Cats

106 and 302 were older and on the basis of previous work would be expected to have lower grade infestations. Two explanations may be given for the unusually high percentage in Kitten 339. In a series of infestations with this strain in cats reported previously (Scott, 1928) none were as high as this but two were so peculiarly high as to indicate that certain animals may be especially susceptible. This is the probable explanation, but it should be recorded that this entire litter had to be returned for two days to a single cage after a period of observation usually long enough to assure that no vomiting would occur. The control animal in this litter had no adult hookworms when autopsied at the same time as the others, but examination of the intestine with the Baermann isolation apparatus revealed that 26 undeveloped larvae were present. This indicates that some animals may have vomitted some larvae which were then eaten by the control kitten. This opens the possibility of other larvae having been eaten by the other kittens in the same way. The results of the other experiments would lead to the same conclusions if this litter were left out of consideration altogether, but it should be recorded on the assumption that Kitten 339 may represent an extreme but normal variation.

DISCUSSION

The literature reviewed above shows that *A. caninum* is probably not as common in cats as it is in dogs, but is recorded in this host for a number of places well distributed over its range in dogs. The indications are that as a rule it produces smaller infestations in cats than in dogs. There is also some definite evidence that in a few localities at least it does not occur in cats where it is common in dogs. The facts of the experiments described here offer an explanation of this condition. If the dog strain used here is representative of the infectivity for cats of most dog hookworm, one would expect it to occur only occasionally in this host and then only in small numbers. Where a strain similar to the cat strain used here has been developed locally one would expect to find more and larger infestations in cats. Work is now in progress to test other strains from cats in localities where it commonly occurs in this host. The cooperation of anyone who is able to supply material from cats in such a locality or from dogs in a locality where the parasite does not occur in cats would be greatly appreciated.

Another situation, which may be similar to this and which should be tested by the same methods concerns another genus of this same family of parasites. Hall (1918) and Ransom (1924) mention the fact that *U. stenocephala* is common in dogs of Europe but has been reported from this host only once in this country, although it is a common pest in the fur fox farms. The single report of its occurrence in dogs in the United States (Muldoon, 1916) is not entirely clear since, as Hall

mentions, the specimens were not preserved and there is a possibility of misidentification. An adequate explanation of this distribution would be offered if two strains of this parasite could be demonstrated as especially adapted to the dog and to the fox respectively.

A demonstration of the occurrence of strains such as those described here in a number of species of common parasites would help to explain some puzzling facts of distribution and epidemiology.

SUMMARY AND CONCLUSIONS

1. A strain of the dog hookworm, *A. caninum* (Erc. 1859), obtained from a cat appears to be especially adapted to this species of host. This is evident from the fact that an average of 45 per cent of the larvae matured in young kittens while less than one per cent matured in puppies.

2. The strain of the same species of parasite obtained from a dog shows opposite degrees of infectivity to these two hosts. An average of 50 per cent of the larvae matured in puppies while less than 5 per cent matured in kittens.

3. The experiments demonstrate two strains of a parasite morphologically identical but physiologically different in their adaptation to different hosts. This proves what has been suggested or partially proved for other parasites. The findings may prove to be an explanation of the peculiarities of distribution and epidemiology of this and other parasites in various hosts.

Literature Cited

Adler, S. 1922.—Ancylostomes in animals in Freetown. Ann. Trop. Med. Parasit., 16: 293-294.

Chandler, A. C. 1925.—The helminth parasites of cats in Calcutta and the relation of cats to human helminth infections. Ind. Jour. Med. Res., 13: 213-227.

Faust, E. C. 1921.—Preliminary survey of the parasites of vertebrates of North China. China Med. Jour., 35: 196-210.

Gordon, R. M. and Young, C. J. 1922.—Parasites in dogs and cats in Amazonas. Ann. Trop. Med. Parasit., 16: 297-300.

Hall, M. C. 1917.—Parasites of the dog in Michigan. Jour. Amer. Vet. Med. Assn., 51: 383-396.

1918.—A Physaloptera from the dog, with a note on the nematode parasites of the dog in North America. Jour. Amer. Vet. Med. Assn., 53: 733-744.

1928.—Parasitological and other observations in Central America. Cornell Veterinarian, January, 1928.

Johnston, T. H. 1916.—A census of the endoparasites recorded as occurring in Queensland, arranged under their hosts. Proc. Roy. Soc. Queensland, 28: 31-79.

Maxwell, J. P. 1921.—Intestinal parasitism in South Fukien. China Med. Jour., 35: 377-382.

Muldoon, W. E. 1916.—Uncinariasis in dogs. Rept. N. Y. St. Vet. Coll., 1914-1915: 136-141.

Ortlepp, R. J. 1926.—On a collection of helminths from a South African farm. Jour. Helm., 4: 127-142.

Ransom, B. H. 1924.—Hookworms of the genus Uncinaria of the dog, fox and badger. Proc. U. S. Nat. Mus., 65: 1-5.

Schwartz, B. 1924.—Report to the Helminthological Society of Washington. Jour. Parasit., 11: 93.

1926.—Parasitic nematodes from China. Proc. U. S. Nat. Mus., 68: 13: 1-10.

1926a.—Specific identity of whipworms from swine. Jour. Agri. Res., 33: 311-316.

Scott, J. A. 1928.—An experimental study of the development of *A. caninum* in normal and abnormal hosts. Amer. Jour. Hyg., 8: 158-204.

Tubangui, M. A. 1925.—Metazoan parasites of Philippine domestic animals. Phil. Jour. Sc., 28: 11-37.

Wharton, L. D. 1917.—The intestinal worms of dogs in the Philippine Islands. Jour. Parasit., 4: 80-82.

Wolfflügel, K. 1911.—Los zooparasitos de los animales domesticos en la Republica Argentina. 108 pp., Buenos Aires, 1911. Revista Centro Estud. Agron. Vet., 1910-1911.

SOCIETY PROCEEDINGS

AMERICAN SOCIETY OF PARASITOLOGISTS, FOURTH ANNUAL MEETING, NEW YORK CITY, DECEMBER 27-29, 1928

The program was full of interest and representative of the various lines of research that are being followed out by American parasitologists. A series of demonstrations given on Thursday afternoon was made the occasion for a delightful social hour for the whole Society. The luncheon of the Society was one of the features of the program, with an attendance of about sixty. Only the record of the annual business meeting is given here.

The following preliminary report of the Treasurer for 1928 was read and approved; it was then referred to the Council for audit.

Balance from 1927	$ 51.24
1926 and 1927 dues paid in 1928	15.00
1928 dues, from 401 members	401.00
Total amount available for 1928	467.24
Expenditures for 1928	409.66
Balance available for 1929	$ 57.58

The report of the Secretary showing the present status of membership was read and approved. It is as follows:

	United States	Foreign Countries	China Branch	Total
Members in good standing, paid for 1928 or 1929	362	60	9	431
Applicants for membership	5	..	..	5
Members delinquent for 1928	42	7	29	78
Members delinquent for 1927 and 1928	9	6	13	28
Total membership to date	418	73	51	542
Members lost by death, resignation and dropping	18	5	..	23
Total elected to membership since organization of Society	436	78	51	565

A report of progress was given by the committee on making the Journal of Parasitology the official organ of the Society. The report was accepted and the committee continued.

A very interesting report of the development and present condition of the China Branch was made by its former president, Dr. E. C. Faust.

It was voted that the Council of the Society be asked to consider the formation of a group of biological or zoological societies that would plan to hold some meetings separately from the American Association for the Advancement of Science, preferably on the campus of some college or university.

The Council, acting as nominating committee, made the following nominations:

President, N. A. Cobb.

Vice president, G. R. LaRue.

Council member for 2 years, F. C. Bishopp.

Council members for 4 years, C. W. Stiles, A. C. Chandler.

The report of the committee was accepted and the unanimous ballot of the Society was cast for the names proposed. The meeting was then adjourned.

W. W. Cort, Secretary-Treasurer.

PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

One Hundred Eleventh to One Hundred Fifteenth Meeting

The one hundred eleventh meeting was held March 17, 1928.

The following were elected to membership:

(Foreign) Dr. J. E. W. Ihle, Dr. Sandor Kotlan, Dr. J. G. de Man, Dr. E. Martini, Dr. Teodor Odhner, Prof. K. Skrjabin, Prof. Warrington York. (American non-resident) Dr. William Taliaferro, Dr. Seymour Hadwen. (Active resident) Dr. Mario Mollari.

Dr. N. R. Stoll presented a report on his experimental infections with the twisted wireworm, *Haemonchus contortus,* in sheep. Utilizing as hosts the nearly helminth free animals described by Smith and Ring (Jour. Parasit., 13: 260, 1927), and as technics both dilution and floatation counts it was possible by computing the egg output to follow changes in the parasite burden of animals placed upon plots under conditions where reinfection occurred. This type of study resulted in the rather striking discovery of a self-cure phenomenon on the part of the hosts, evidently through elimination of the parasites. Moreover, animals which had gone to a self-cure were protected almost completely against reinfection. The possibility was advanced that perhaps certain special conditions of the experiment produced this effect, although there are numerous anomalous observations in the literature bearing not only on Haemonchus infection, but on other gastro-intestinal nematodes as well (see for instance, Herrick: Am. Jour. Hyg., March, 1928), which are most easily harmonized with this conception of the intervention of self-cure and protection on the part of the host. The more likely possibility, therefore, is that there occurs in nematode infections of the type of *H. contortus,* the development of a resistance, which is independent of an age resistance per se, and which acts to protect the host, to the same end (and which may or may not have the same modus operandi) as obtains for typical bacterial infections. This phenomenon appears to come about when the host has received a certain quantity of infection, at perhaps not too rapid a rate. If the worms reach the host in small numbers, or in a single dose, a low grade infection results which may or may not produce the effect. Dr. Stoll reported no lethal infections.

Miss Gertrude H. Merrill, R. N. of the American Board Mission Hospital, Mount Silinda, South Rodesia, Africa, reported on the work of this institution as related to helminthology. During the past four years all patients of the hospital as well as many out-patients and employees have been examined for parasites. As a result frequent ascarid infections have been found, mostly among children, and a few other nematodes including Strongyloides, a few cestodes and a few cases of *Schistostoma japonicum.* About half of all those examined harbored hookworms. One four-year old native boy, brought to the hospital for treatment, had the appearance of "a full-stuffed sack, with sticks attached at the corners and a head on top." His color was ashen gray, he vomited often, and suffered frequent spasms of severe pain. On treatment with an anthelmintic 365 ascarids were passed, after which the child recovered. The case is represented by Specimen No. 12354, U. S. Hygenic Laboratory.

The one hundred twelfth meeting was held April 21, 1928.

Dr. N. A. Cobb presented the following notes:

1. On conspicuous amphids in *Physaloptera turgida* from the opossum.

2. *Spiroxys amydae* n.sp. $\frac{0.53(?)}{0.52(?)}$ ..... $\frac{2.6}{2.6}$ ..... $\frac{17.}{2.2}$ ..... $\frac{+50(?)}{+52(?)}$ ..... $\frac{90+}{18+}$ are the measurements of an immature female. The rather thick layers of the

transparent, colorless, naked cuticula are traversed by transverse striae, 8μ apart, readily resolvable with moderate powers. The striae, which are not further resolvable, are interrupted at both margins of the wings, which seem to be only very slightly elevated, if at all. The longitudinal chords are believed to have about the same width as the wing region—probably one-sixth to one-fifth as wide as the body. Anteriorly and on most of the body the contour is very minutely serrate, in a retrorse manner, but posteriorly it is crenate. The cylindroid esophagus is about half as wide as the corresponding portion of the neck; it is separated from the intestine by a definite constriction. The lining of the esophagus is a distinct feature and finds its optical expression in a number of refractive narrow elements passing along the axis of the organ in a somewhat irregular manner. Nuclei are to be seen in the esophagus, each about one-sixth as wide as the organ and removed from each other a distance equalling about one and one-half times their diameter; these nuclei are refractive and apparently exist in three longitudinal rows. They constitute a quite conspicuous feature of the esophagus in the preserved specimen. Near the middle of the body the cuticula is 10μ thick, the subcuticula 8μ thick, and the muscular layer 17μ thick. In the specimen under examination, in the posterior portion of the body, the contour is very coarsely crenate, each crenation comprising anywhere from six to eight of the annules marked off by the cuticular striae. Whether this is due to the method of fixation or not is not known.

As only young female specimens have been examined, it is impossible to give data with regard to the details of the vulva, the vagina and the terminal portions of the gonad. Of course, no eggs have been seen. The excretory pore lies in the midst of a somewhat depressed area and constitutes a readily recognizable feature of the nema. There appears to be a rather distinctly developed, though somewhat small, three-liped cardia.

It would seem that both the tissue and the contents of the intestine must be very finely granular. Unfortunately the specimens from which the description is derived are poorly preserved.

$$\frac{0.58}{0.59}\ldots\frac{2.75}{1.2}\ldots\frac{21.}{1.6}\ldots\frac{M}{2.4}\ldots\frac{99.}{4\ 1.1_{\frac{7}{8}}}\ 23.2\text{mm}$$

These are considered fairly reliable measurements of the male.

Habitat: In subspherical saccate lesions in the wall of the stomach of the soft-shelled turtle, *Amyda ferox,* of the Mississippi River, *fide* G. A. MacCallum.

Diagnosis: Spiroxys, dimensioned as shown in the formulae and illustrations. Pharynx with eight odontia. Spicula equal, nearly parallel. Main bursal ribs, three; about 11 pairs of papillae in all, of two different sizes.

3. In a paper presented before the Helminthological Society I had given the name of a certain nema as *Physaloptera phrynosoma* Ortlepp 1922. In a recent paper Schulz, as a result of later studies of this species and its relatives, places the species in the genus *Skrjabinoptera,* under the name *Skrjabinoptera* (*Didelphysoma*) *phrynosoma* (Ortlepp 1922).

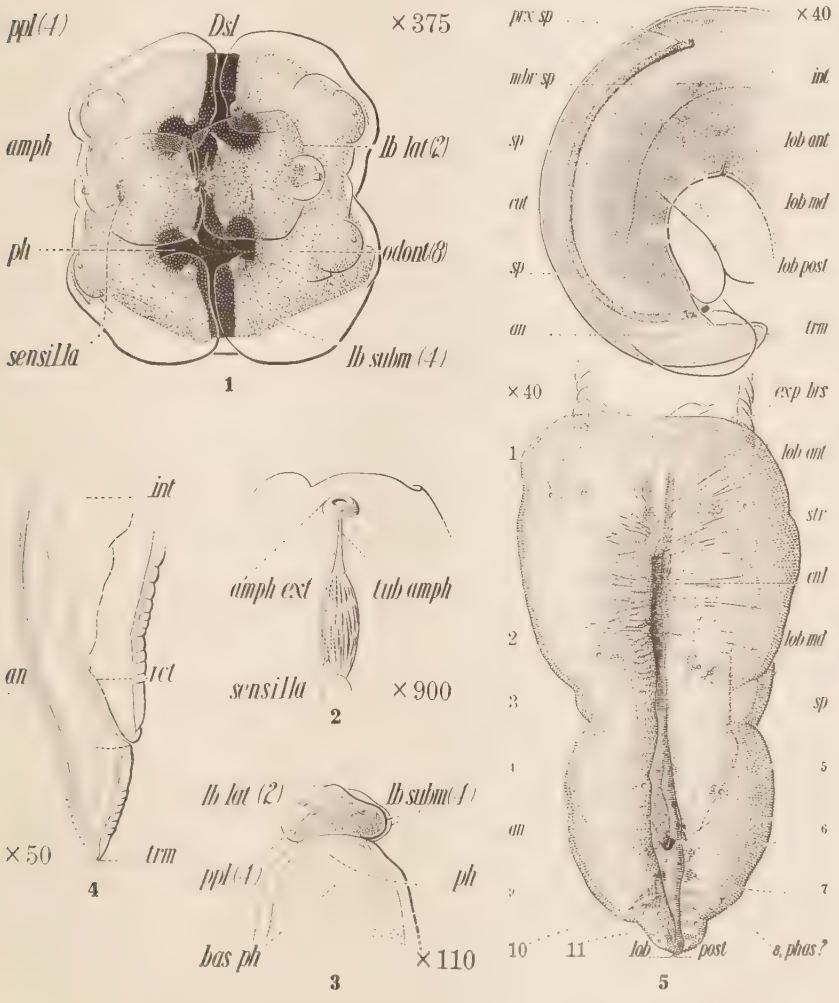
Drs. G. F. White and W. E. Dove reported as follows on the results of their investigation of creeping eruption: Earlier we reported that the symptoms and advancing linear lesions characterizing creeping eruption had been produced by applying to the human skin infective larvae cultured from the dog and the cat infested with *Ancylostoma braziliense* and *A. caninum.* A little later we announced the production of these symptoms and lesions with pure cultures of *A. braziliense.*

In our early infection experiments using mixed cultures of these two hookworms there were produced, in addition to the linear lesions of creeping eruption, itchy papular ones which disappeared in approximately a fortnight. Naturally it occurred to us that these might have resulted from the invasions of the skin by larvae of *A. caninum.* At this time we are pleased to report some further observations in this connection.

By applying pure cultures of *A. caninum* to the skin of two volunteers, we have produced itchy, papular lesions that were not accompanied by the linear ones of creeping eruption, and which for the most part disappeared before the end of the third week. The cultures used were obtained from animals from three widely

distant localities. An infection of the human skin with larvae of *A. caninum* is itself naturally a disease of much interest. It is at once apparent that it is also of much importance in the diagnosis and treatment of creeping eruption.

In discussion Dr. White recalled that *A. caninum* has, in a few instances, been reported from man. He stated that, when applying the larvae to the skin, no



special precautions were taken to prevent bacterial infection but doubted that the penetration of the larvae brought about any active infection of this kind. Dr. Cobb pointed out the possibility that some nemas, when boring their way through the tissues of the host, sterilize the wounds thus produced.

Dr. E. W. Price presented the following notes:

1. The occurrence of *Cooperia bisonis* Cram in cattle.

Among some nematodes collected from a calf by H. D. Port of Cheyenne, Wyoming, and forwarded to the Bureau of Animal Industry for identification by

Geo. W. Stiles, Denver, Colo., were a few specimens of *Cooperia bisonis*. This is the first report of this species in cattle in this country, it being originally described from buffalo from Wainwright, Alberta, Canada.

2. The generic status of *Distomum gastrocolum* Leidy.

Leidy in 1891 briefly described a trematode from the skipjack (*Trichiurus lepturus*) which he named *Distomum gastrocolum*. Up to the present time this species has not been ascribed to any of the recognized trematode genera. Recently the writer has examined some specimens of the original material and finds that this species is an appendiculate distome of the genus *Brachyphallus* Odhner. In most respects this species closely resembles *B. crenatus* (Rudolphi), but may be differentiated on the basis of the suckers. In *B. crenatus* the suckers are about equal in size; in *B. gastrocolum* the oral sucker is about one-half the size of the acetabulum.

Dr. Benjamin Schwartz exhibited specimens of fly larvae belonging to the genus Sarcophaga which were forwarded to the Bureau of Animal Industry by Dr. Rollo B. Hill from a case of cutaneous myiasis in man in Venezuela. Cutaneous myiasis in man due to these fly larvae is rather common in Venezuela according to information furnished by Doctor Hill.

Dr. Paul Barsch stated that in an examination of the students of Howard University for hookworms, only four out of 63 were infected, and those light cases. This represents a decided decrease from the results of a similar examination a year ago. This decrease in the incidence of hookworm infestation is attributed by Dr. Bartch to the fact that the medical school is drawing its students in increasing numbers from city communities.

The one hundred thirteenth meeting was held at Dr. Stiles' residence on May 12, 1928.

The following were elected corresponding members: (Foreign) Prof. J. Fiebiger, Dr. I. N. Filipjev. (American non-resident) Prof. Aldo Castellani.

Dr. G. Steiner, in describing a new species of Acrobeles, commented on the probable function of the labial and cephalic probolae. This genus, related to Cephalobus and Rhabditis, is believed to feed on decaying organic matter and the probolae may fold over the mouth in such a way as to function as a sieve and aid in the selection of food. (Details to be published elsewhere).

Dr. Frank G. Brooks reported on the germ cell cycle in Trematodes. (Published in Science 68: 277-278.)

Miss Myrna Jones reported a new locality and a new host for *Schistotaenia macrorhyncha* (Rud., 1810) Cohn, 1900. The specimens were collected from *Podilymbus podiceps* in Brazos County, Texas, by E. W. Price. So far as is known the cestode has not been reported from this country, nor from this host, although known in other members of the grebes, the Colymbidae, in Europe.

The large scolex and rostellum (Fig. 6) distinguish *Schistotaenia macrorhyncha* from other genera of Amabiliidae. The large hooks (Fig. 7) which are easily lost in spite of the elaborate musculature of the rostellum vary in size from 128 to 150μ long, with a ventral root from 78 to 80μ long. In the one specimen with hooks, 23 hooks were observed clinging in 2 groups to the scolex; probably the total number was at least 24 or 26. While the hooks of this one specimen which had hooks are larger than those of Cohn's material (105μ long), they are comparable in size to those of Rudophi's material, which from the illustrations of Cohn's paper are apparently 145 to 150μ long.

Another point of interest concerns a generic character, the irregular alternation of the male genital pores. In a strobila of 95 segments there were only three regions where irregular alteration could be observed; in the remainder of the strobila the pores alternate regularly.

Dr. N. A. Cobb presented a communication from Mr. Gerald Thorne dealing with the nemic fauna at the summit of Longs Peak. (To be published elsewhere.)

Dr. Cobb also called attention to the fact that *Tylenchus dipsaci* appears to be slowly spreading in the United States. Diseased plants have been sent to the

Department of Agriculture from the following localities where it has not previously been known to exist:

Narcissus bulbs: Scottsville, Texas; Tillamook, Oregon; Takoma Park, D. C.; Arlington, Va. (two lots); College Park, Md.; Raleigh, N. C. (heavy infestation); Columbus, Ohio; Urbana, Ill.

Alfalfa: Stillwater, Okla.; various parts of Oregon.

Iris bulbs: Philadelphia, Pa.

Primula: Harrisburg and Willow Grove, Pa. (heavy infestations).

Teasel: Molalla, Oregon (heavy infestation).

The one hundred fourteenth meeting was held October 20, 1928.

The following were elected active members: Mr. B. G. Chitwood, Mr. J. E. Stumberg, Dr. D. Sinitsin, Mr. Joseph Alicata, Miss Mary Skinker, Dr. W. H. Wright.

Capt. Charles S. Butler was elected president.

Dr. J. A. Scott reported (supplementing a previous report, Jour. Parasit., 14:197) concerning a strain of dog hookworm, *Ancylostoma caninum*, to which the cat is especially susceptible. It will be recalled that a relatively large per-

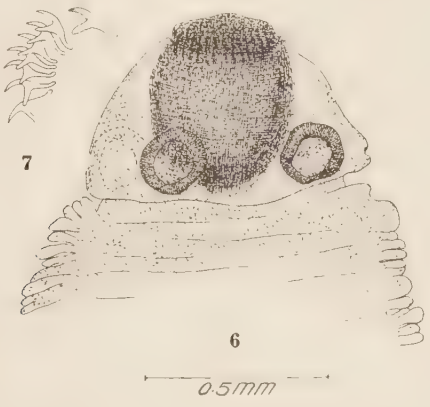


Fig. 6.—Anterior portion of *S. macrorhyncha* showing head.

Fig. 7.—A portion of a crown of hooks from *S. macrorhyncha*.

centage of infective larvae of the dog strain developed in dogs and of the cat strain in cats. A very small percentage, however, developed in the host other than the one from which the strain was derived. Data on the growth, as obtained by measurement of photographs of a large series of worms removed at autopsy, show a pronounced effect of the host on the growth of either strain. Both the dog strain and the cat strain grew more slowly in cats than in dogs and also failed to attain as great a final size as those in the latter host. Both strains grew at the same rate and attained approximately the same final size in a given host. (Details to be published elsewhere.)

Dr. C. W. Stiles and Mrs. C. E. Baker reported a fifth case of *Gongylonema hominis* in man in the United States. (To appear in Jour. Am. Med. Assoc.)

Doctor Sinitsin presented the following notes on larval trematodes which were found by him during July-September, 1928, in the vicinity of Washington, D. C.

1. *Cercaria aurora* sp. nov. This form developed in simple sporocysts which occur in the liver of *Physa heterostropha*, a snail which was found in large numbers in a pool near Aurora Hills, Va. The body is 0.25 mm. long by 0.12 mm. wide and the stylet is 0.024 mm. long. This cercaria belongs to a type similar to *Cercaria brevicocca* Cort. They readily attack tadpoles in which they become

encysted. Some features of the anatomy of the adolescaria indicate that *Cercaria aurora* is the larval form of *Renifer acetabularis,* a parasite of *Natrix rhombifera.* Drawings of this species were exhibited.

2. Land-snails were collected in the vicinity of Washington, D. C., were found to be infested with several species of larvae belonging in the family Harmostomidae. The following species of land-snails were examined: *Polygyra thyroides, P. tridentata, Gastrodonta suppressa, G. ligera,* and *Helicodiscus parallelus.* The adolescariae of one of the species were found only in the kidneys of their hosts, while the adolescariae of the other species were encountered only in the pericardium. The well developed genitalia of the adolescariae permit identification of the species. Drawings of four species of cercaria from land-snails were exhibited.

3. A new species of cercaria was found in a specimen of *Goniobasis rubicunda* from California. In this form the ventral sucker is placed at the side of the body. This species has no tail and its adolescarial stage occurs in the same snail host. The snail and drawings of the cercaria were demonstrated.

4. In water snails, *Galba bulimoides techella,* which were collected by Doctors M. C. Hall and G. Dikmans from a pasture at Jeanerette, La., where cattle were heavily infested with *Fasciola hepatica,* radiae and cercariae of this fluke were found. The cercariae which escaped from the infested snails were found encysted on the walls of the aquarium and on the surface of the water. In cooperation with Dr. E. W. Price, some feeding experiments were undertaken. A sheep and several rabbits were fed with the cysts. After 2, 4, and 7 days young *Fasciola hepatica* were found in the abdominal cavity of the rabbits. Experiments to infest snails with miracidia of this species which were hatched in the laboratory were undertaken. Positive results were obtained with *Galba bulimoides techella.* In this snail the miracidia developed in the same manner as described by both Thomas and Leuckart for the European liver fluke. *Galba desidiosa modicella* and *Physa heterostropha* appeared to be immune.

In connection with this note Dr. Sinitsin demonstrated (1) drawings and photographs of the larval stages of *Fasciola hepatica,* (2) slides of young distomes from experimentally infested rabbits, (3) slides of miracidia and young mother sporocysts, (4) aquarium containing living *G. bulimoides techella,* and (5) living miracidia of *F. hepatica* attacking *G. bulimoides techella* and at the same time ignoring the other species of snails which were placed in the same aquarium.

Dr. W. W. Cort discussed further observations on Schistosome Dermatitis (see Science, 68: 388, 1928).

The one hundred fifteenth meeting was held at the School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md., November 17, 1928.

The following were elected active members: Mr. Benjamin Collins, Miss Corinne Cooper, Mrs. Dorothy Cobb Adams, Mrs. D. Sinitsin, Dr. Benjamin Levine, Dr. George W. Bachman, Mr. John Bozevich.

Dr. M. C. Hall summarized a paper on the Arthopod hosts of Helminths (to appear in Proc. 4th Entomological Congress at Ithaca, N. Y., 1928).

Dr. D. Sinitsin presented the following note: Parthenitae and cercariae which appear to belong to *Telorchis robustus,* a trematode parasite of *Cistudo carolina,* were found in the liver of *Polygyra thyroides.* The parthenitae have the form of simple, cylindrical sporocysts which measure about 5 mm. long by 0.5 mm. wide. The cercaria measures 0.4 mm. long by 0.13 mm. wide; the tail is slightly longer than the body and is inactive due to the poorly developed musculature. The stylet measures about 0.015 mm. in length. There are nine pairs of stylet glands which lie caudad of the acetabulum. The oral sucker is larger than the acetabulum, and is elongated anterior-posteriorly; acetabulum situated near equator of body. The excretory vesicle is Y-shaped, the stem being long and S-curved. The disposition of the genitalia is that of the Telorchidae. This species was found only once in 400 specimens of *Polygyra thyroides* examined. These snails were collected in a wood near Four Mile Run, Va. A drawing of the cercaria was exhibited.

Dr. Benjamin Schwartz presented the following notes:

(1) The occurrence of nodules in the wall of the fourth stomach of sheep due to *Ostertagia ostertagi*. Although this worm which occurs in nodules in the wall of the fourth stomach and also free in the cavity of the fourth stomach of cattle has heretofore been reported from sheep, the records are open to suspicion because the reports are not based apparently on a morphological study of the parasites. Ransom (1911) says: "This species has been frequently reported by various authors as a parasite of sheep, but in most instances such reports have evidently resulted from a confusion with *O. circumcincta*. Among hundreds of specimens of species of Ostertagia from domestic sheep, mostly collected in this country, which I have examined, I have failed to find *O. ostertagi*."

In the course of postmortem examinations of a number of sheep which were shipped from the Mississippi Agricultural and Mechanical College to Beltsville, Md., for slaughter, nodules were found in the wall of the fourth stomach. Several of the nodules examined contained a nematode which proved to be morphologically identical with *Ostertagia ostertagi*.

Dr. Price informs me that on several occasions he had noticed nodules in the wall of the fourth stomach of sheep slaughtered at Beltsville, Md., but that he had failed to find nematodes in them.

(2) The occurrence of Ascaris in the bile ducts of sheep. Under date of May 24, 1928, there were forwarded to the Bureau of Animal Industry from the National Stock Yards, Illinois, specimens of Ascaris collected from the bile ducts of five sheep. Additional specimens of Ascaris collected from the bile ducts of several sheep were received from the same abattoir on June 9 and 13, 1928. The material from the last shipment contained numerous specimens, of which 20 were collected from the bile duct of one sheep. In this case the origin of the sheep was traced to Missouri, but the exact locality could not be determined.

Although the specimens were incompletely grown as compared to the size attained by the swine and human ascarids, several females contained eggs which were cultured in a ½ per cent solution of formalin and in the course of about two weeks became embryonated. This confirms Goodey's observation that Ascaris may develop to fertile maturity in sheep. One might raise the question as to whether a strain of swine ascarids biologically adapted to sheep is being developed.

(3) A new case of Dipylidium in man. Under date of August 16, 1928, Dr. James M. Barnett of Albany, Va., forwarded to the Bureau of Animal Industry gravid segments of tapeworms passed by a child, 2 years old. Dr. Barnett stated that the segments had been observed in the child's feces for a period of two months. Upon examination the segments proved to be a species of Dipylidium.

In connection with this new record of Dipylidium, Dr. Schwartz presented a note for Dr. W. H. Hoffman of the School of Tropical Medicine at San Juan, Porto Rico, calling attention to the first case of Dipylidium in man from Porto Rico, the subject being a child, 2 years old.

Dr. G. Steiner discussed a new species of Neodiplogaster which occurs as larvae under the wings of the white pine weevil, *Pissodes strobi*. The adults are found inhabiting the frass in the gallaries of this weevil. (Details to be published elsewhere.)

Dr. N. A. Cobb called attention to recent successful experiments with the hot water treatment of nematized plant roots. In discussion Dr. Steiner pointed out the great economic benefits which will result from the successful application of this treatment.

Dr. Cobb also called attention to the head pigments (phototrope) of *Mermis subnigrescens* females, and discussed their reactions to various light frequencies, and the role this plays in egg deposition. (Details to be published elsewhere.)

Miss Myrna F. Jones reported as follows:

(1) After experimentally feeding gravid segments of *Davainea proglottina* to various molluscs, the cysticercoids of *D. proglottina*, hitherto only known in the slugs *Limax cinereus* and *Agriolimax agrestis*, have been found in four species of

snails—*Zonitoides arborea, Vallonia indentata, Gasterodonta ligera,* and *Polygyra thyroides.* Chickens given infected snails became infected with mature specimens of *D. proglottina,* control birds remaining free, so that each of the species of snails named can serve as an intermediate host for *D. proglottina. Zonitoides arborea* and *Vallonia indentata* have been found about the chicken yards at the Beltsville, Maryland, Experiment Station fairly commonly; *G. ligera* has been found there occasionally, and *P. thyroides,* rarely.

(2) It has been found that another small dung beetle, *Hister* (*Carcinops*) *14-striatus,* can serve as an intermediate host for *Hymenolepis carioca.* One beetle, of a miscellaneous group previously given gravid segments of *H. carioca,* contained several unarmed cystercoids when dissected and was fed to a chicken which, upon post-mortem examination about 3 weeks later, harbored 1 mature specimen of *H. carioca* while control birds remained free. When material is available it is planned to repeat the experiment on a larger scale.

Mr. Oliver R. McCoy discussed the growth of hookworm larvae on pure cultures of bacteria. (Details to appear in Science.)

Dr. Lawrence Getz exhibited specimens and discussed cases of helminthiasis, especially *Ascaris lumbricoides* and *Trichuris trichiura,* which had come under his observation at the Canal Zone.

Dr. C. W. Stiles presented a brief preliminary statement of a paper involving the nomenclatorial and taxonomic history of *Pediculus vespertilionis.*

J. R. Christie, *Secretary.*

BOOK REVIEWS

THIRTEENTH AND FOURTEENTH REPORTS OF THE DIRECTOR OF VETERINARY EDUCATION AND RESEARCH, DEPARTMENT OF AGRICULTURE, UNION OF SOUTH AFRICA. Two parts. Government Printing and Stationary Office, Pretoria, 1928. 1,270 pages, 13 col. pl., many other illustrations.

The impressive report in two volumes is the last of a series, completed before the retirement of the late director, Sir Arnold Theiler. It is a fitting close to the series which bears abundant testimony to brilliant research work in the field of veterinary science. Not merely in size but equally in variety and value of work are the volumes worthy of especial notice. The forty separate articles contained therein cover a wide range of topics and constitute important contributions to knowledge of the field. Of special interest to the student of parasitology are items on tick-borne diseases, on nagaña, on Onchocerca, on Rhinosporidium, and on other internal and external parasites. An extensive and carefully organized check list of Helminthes in domesticated animals in South Africa and a monographic study of South African mosquitos will be of great value to a variety of students. The size of the work precludes more detailed discussion of its individual items. The papers are well illustrated and well printed. Altogether it makes an impressive showing for the Department of Agriculture in the Union of South Africa as well as for the authors and the particular branch of the service represented.

RECENT ADVANCES IN BACTERIOLOGY AND THE STUDY OF THE INFECTIONS J. HENRY DIBLE. P. Blakiston's Son and Co., Philadelphia, 1929.

This little volume of 363 pages is one of a series on "recent advances" in certain of the medical sciences. The author has rather strictly adhered to this idea. Among topics discussed which would be of special interest to parasitologists are: bacteriophage, experimental epidemiology, filterable viruses, the encephalitis-herpes problem, rabies, vaccinia-variola, typhus fever, trench fever, Rocky-mountain spotted fever, heartwater, tularemia, spirochaetal infections, infective jaundice, etc. The author has not only presented the recent advances on old subjects but has also introduced new subjects. It is interesting to note that in Chapter I, he presents an outline for classification of bacteria prepared by a committee of the Society of American Bacteriologists as "adopted by the Society of American Bacteriologists." Although there is disagreement among the members of this organization with regard to the status of this classification, it is quite generally agreed that it shall be called simply the "Committee's Classification." As Professor Dible stated, this classification is creeping steadily into usage and its presentation in the book seems to the reviewer to be quite appropriate. The discussions of the subjects treated are condensed as they would have to be in a book of this length but yet sufficiently complete. At the end of each chapter is a short bibliography. More attention should have been given to preparation of the illustrations by the publishers. Some of them should have been redrawn and relettered. The book is a contribution and should be of special interest to busy practitioners or others who desire to keep abreast of progress in this rapidly advancing field of knowledge.

RESEARCHES IN POLYNESIA AND MELANESIA. HUMAN DISEASES AND WELFARE. By PATRICK A. BUXTON. Parts V-VII. The London School of Hygiene and Tropical Medicine, 140 pp., 27 pl., 1928.

This account of investigations in Samoa, Tonga, the Ellice Group, and the New Hebrides, in 1924-1925, contains a long section on studies in filariasis with a briefer section including short references to a dozen other diseases. Thus evidently and with right, emphasis have been laid upon the preëminent importance of filariasis as a disease in that section of the globe. Buxton finds that the pathology

of this disease is one of the most complex and least understood problems in tropical medicine. After reviewing the methods used, normal findings, pathological conditions, the distribution and frequence of the complaint, and the carriers responsible for the spread of the parasite, he discusses the results in a summary too extended even to be abstracted here. In this he says "One concludes that filarial infection is an essential precursor to elephantiasis; but the exact position of the filaria in the pathology of elephanthiasis is obscure, and cannot properly be studied in Oceania." The author has included an interesting discussion of the brown man and white in Samoa. Numerous tables and a series of good photographs complete the work.

THE BIOLOGY OF SPIDERS. By THEODORE H. SAVORY. The Macmillan Company, New York, 1928. 376 pp., 16 pl., 121 text figs.

Scientific workers have long had at their disposal abundant and somewhat exhaustive discussions of taxonomic questions concerning spiders but there has been unfortunate lack of biological information regarding these organisms. Moreover such material as has been available is scattered through a wide range of periodicals and often in briefer articles. The difficulty which has been experienced thereby is largely removed with the appearance of Mr. Savory's work. The biological worker finds here in attractive and well ordered form an extended account of spiders with a brief section on related forms like scorpions and mites for comparison. The author gives a discussion of spider bite and spider venom in better form than is available elsewhere in a collective discussion. Unfortunately he has overlooked some important American articles such as that of Baerg. The author's style is attractive, the treatment is generally complete and the work has been presented in attractive form with a good bibliography, but the index is somewhat limited for the convenience of the student.

An important paper has been printed by the *Office International D'Hygiene Publique* on Rodents and Fleas in the Maintainence and Dissemination of the Plague (Masson et Cie, Paris, 1928). It opens with a report by Professor Ricardo Jorge, Director General of Public Health and Delegate of Portugal. This report summarizes the results of an inquiry of the permanent committee. Appendices follow dealing with the work that has been done in various countries with regard to the relation of rodent and fleas to the plague. The closing section of the work is a systematic study descriptive of the principal types of rodent fleas which are responsible for the transmission of plague. This section is written by E. Roubaud, Professor of Medical Entomology, Pasteur Institute.

NECROLOGY

Dr. Joseph Goldberger, U. S. Public Health Service, died January 17, 1929, in Washington, D. C. While his monumental work on pellagra will remain as his greatest service to science and humanity, he made important studies on various topics in animal parasitology, i. e., dengue fever, straw mite disease, head louse and typhus.

Professor Teodor Odhner died October 29, 1928. He was director of the Naturhistoriska Riksmuseum at Stockholm. He was well known for his studies on Trematodes in which he made important contributions to the interpretation of the origin and modifications of the group.